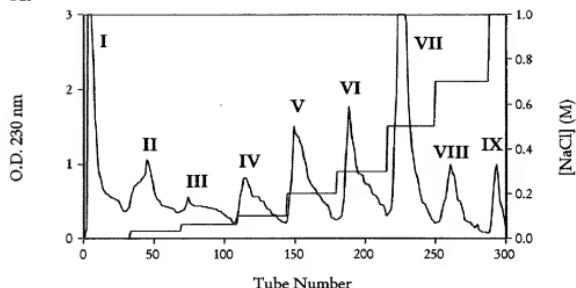
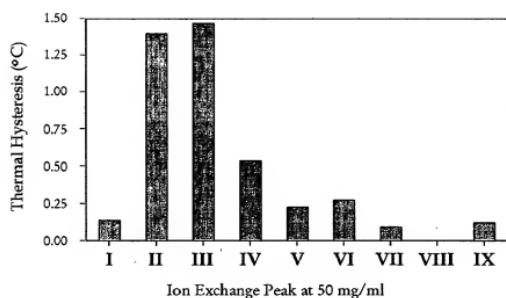


Fig. 1.0

A.**B.***Fig. 1.1*

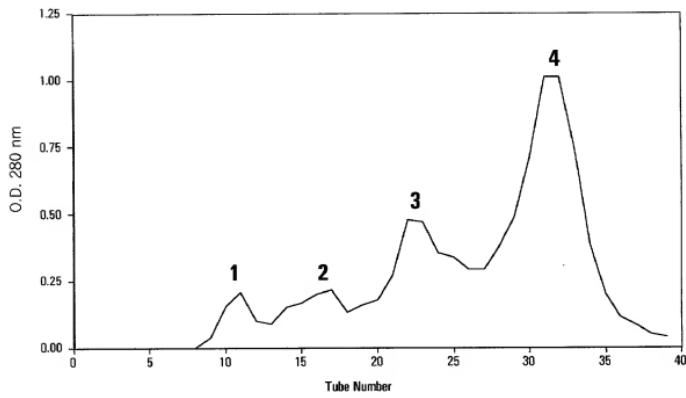


Fig. 1.2

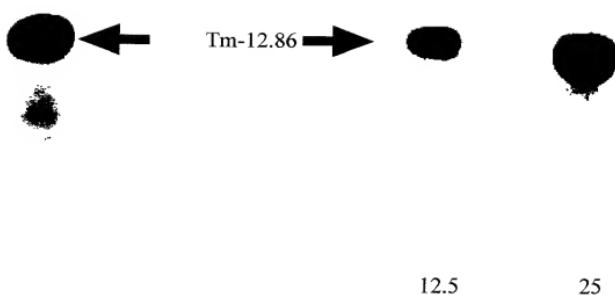


Fig. 1.3

Fig. 1.4

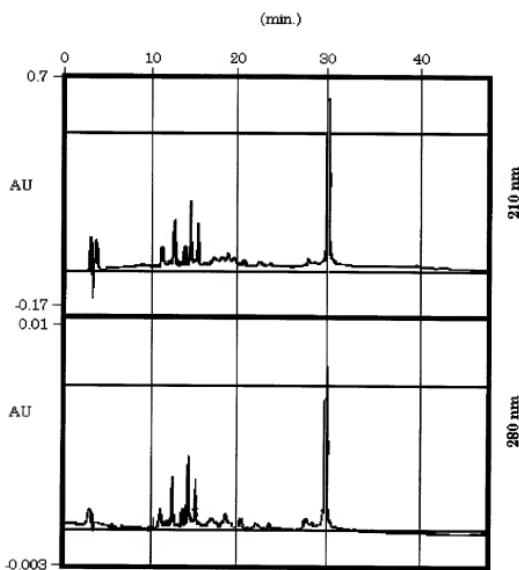


Fig. 1.5

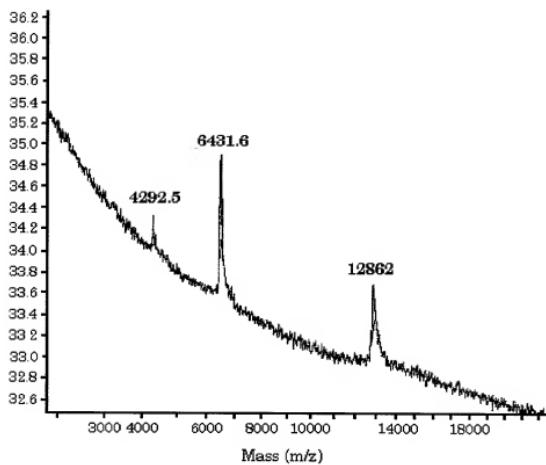


Fig. 1.6

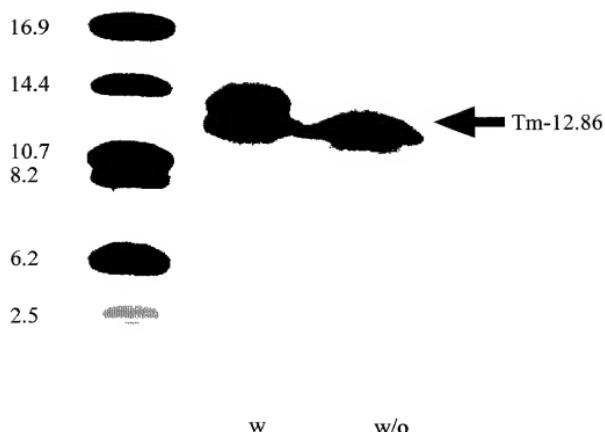


Fig. 1.7

39876796 • 021202

NH₂-L-T-D-E-Q-I-Q-K-R-N-K-I-S-K-E-?-Q-Q-V

Val
Gln
Gln
Gln
Glu
Lys
Lys
Ser
Ile
Lys
Asn
Arg
Lys
Gln
Ile
Gln
Gln
Asp
Thr
Leu

Fig. 1.8

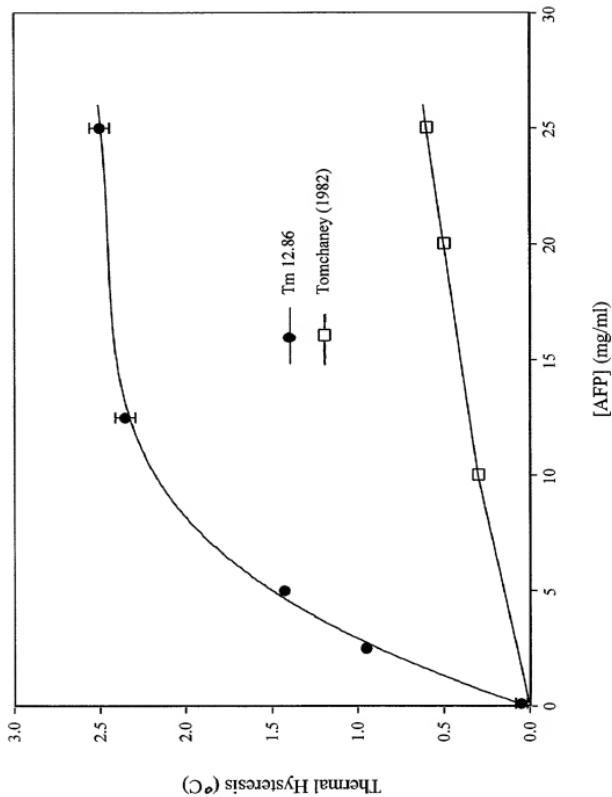


Fig. 1.9

09876796.0021202

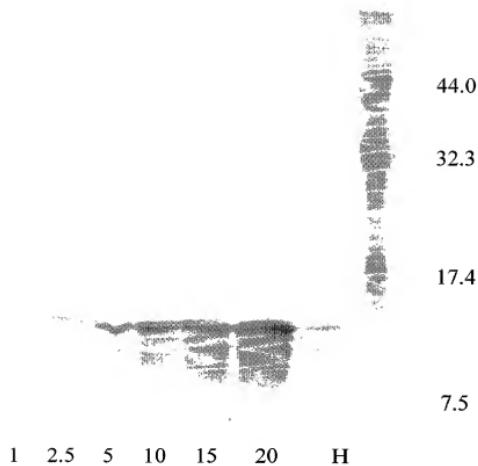


Fig. 1.10

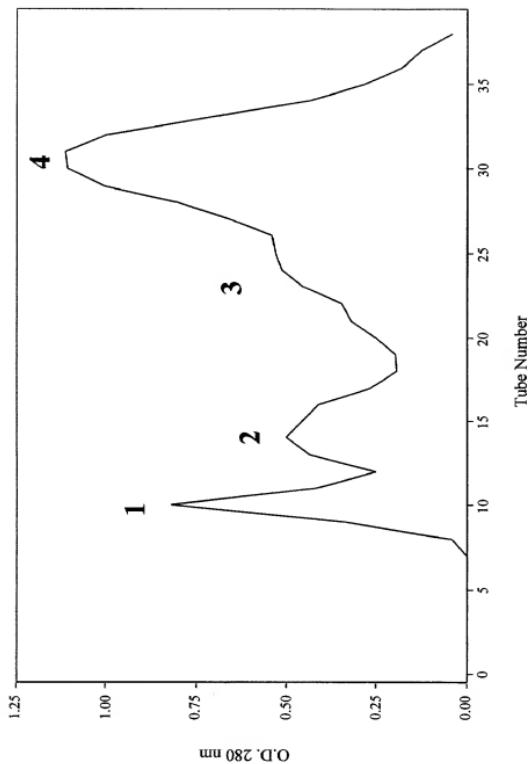


Fig. 1.11

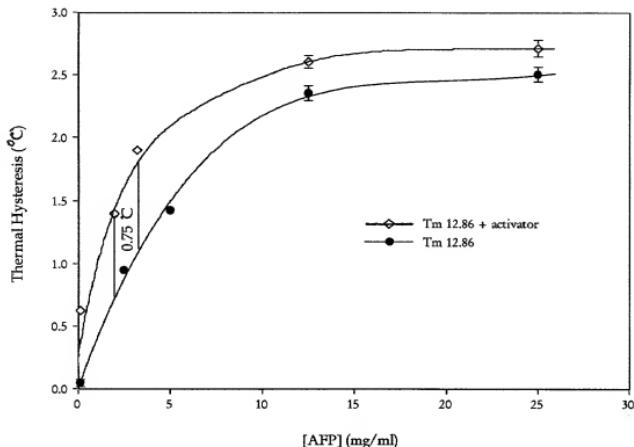


Fig. 1.12

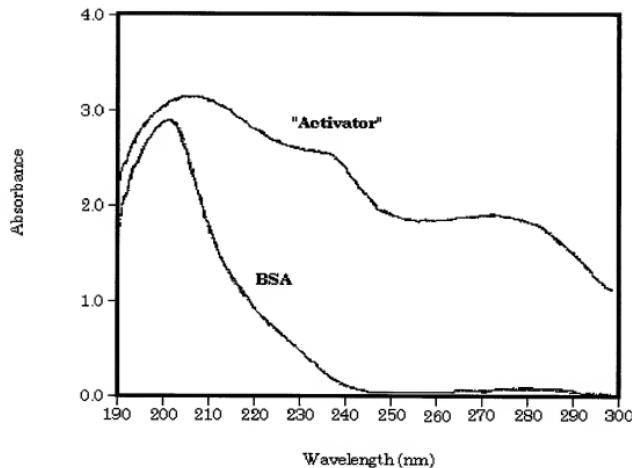


Fig. 1.13

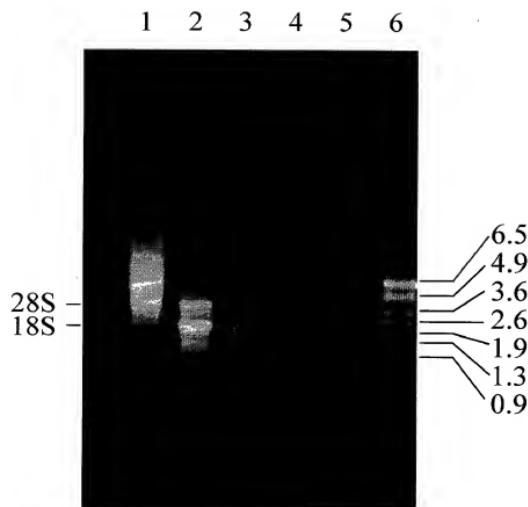


Fig. 2.0

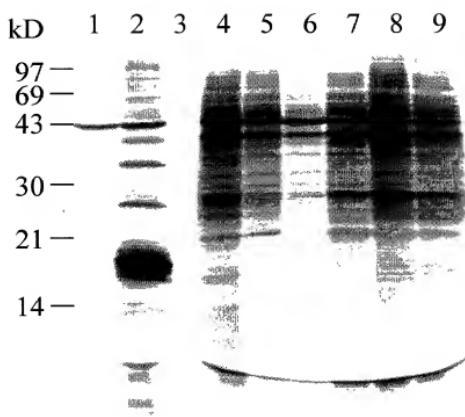


Fig. 2.1

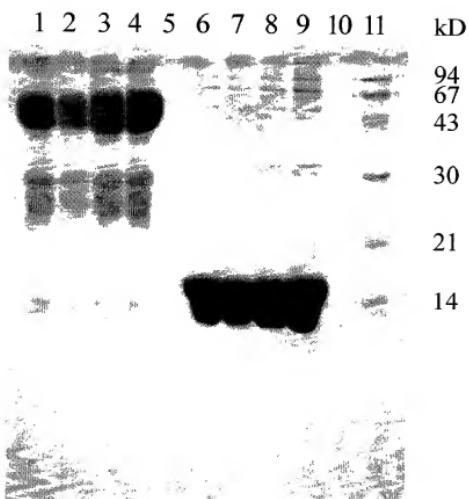


Fig. 2.2

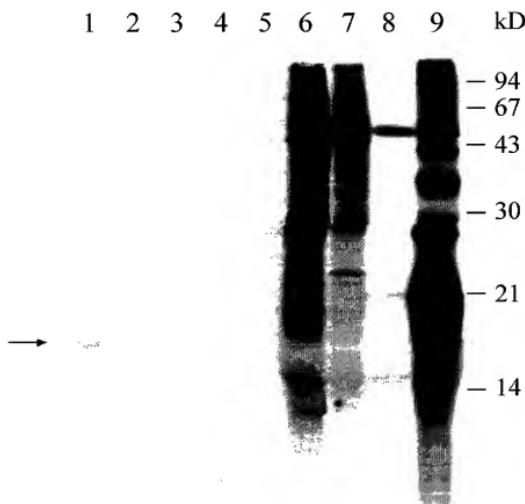


Fig. 2.3

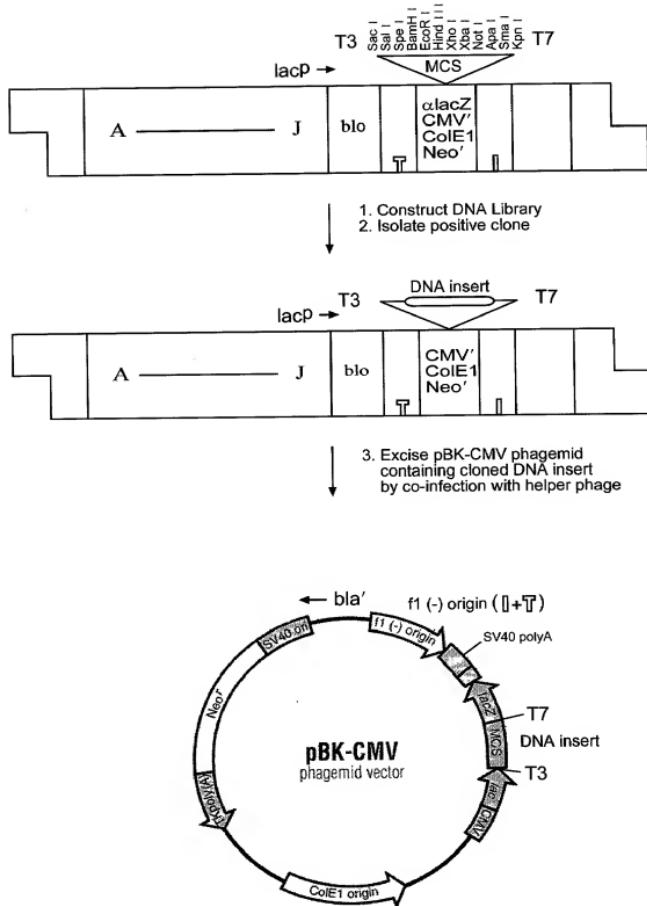


Fig. 2.4a

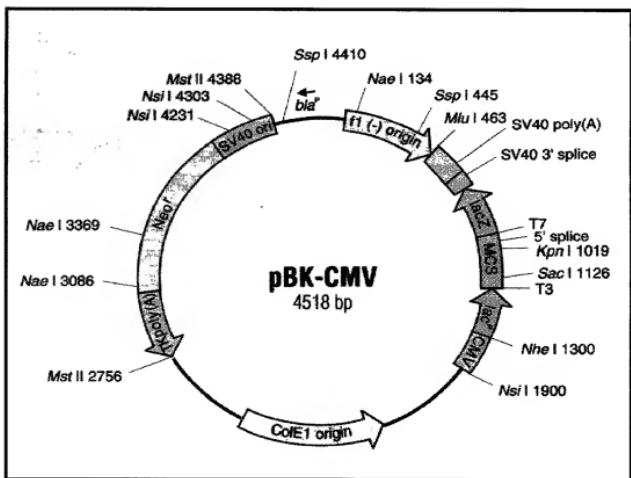


Fig 2.4b

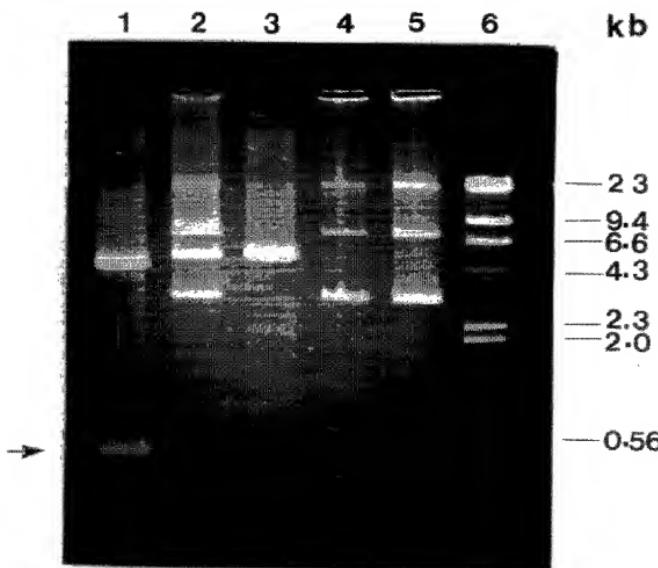


Fig. 2.5

DNA sequence of Tm 13.17 cDNA clone

B	E
a	c
m	o
H	R
I	I

1 AGTGGATCAAAGAATTGGCACGGAGACTACTAAGATGAAAGTTGCTCTGGTCTAAATCT
M K L L C C L I S

61 CCCTCATTCTGTTGGTCACAGTTCAAGGCCCTGACCGAGGCACAAATTGAGAAACTGAACA
L I L L V T V Q A L T E A Q I E K L N K

121 AGATCAGCAAAAATGTCAAAATGAAAGTGGAGTGTGCAAGAGATCATAACCAAAGCTC
I S K K C Q N E S G V S Q E I I T K A R

181 GCAACGGTGAATGGGAGGACGATCCTAAACTGAAACGCAAGTTTTGCGTGGCAGGA
N G D W E D D P K L K R Q V F C V A R N

241 ACGCCGGTCTGGCACGGAATCGGGAGAGGTGGTGGTGCACGTGTTGAGGGAGAAGGTGA
A G L A T E S G E V V V D V L R E K V R

301 GGAAGGTCACTGACAACGACGAAAGAAACTGAGAAAATCATCAAATAAGTGCAGCCCTCAAGA
K V T D N D E E T E K I I N K C A V K R

361 GAGATACTGTTGAAGAGACGGTGGTCAATACTTCAAAATGTGTATGAAAAAACAGCCRA
D T V E E T V F N T F K C V M K N K P K

421 AGTTCTCACCACTGATTGAACCAACACGACTAGTAGATGGTCAATGGTGTGCTTTAC
F S P V D *

x
h
o
i

481 ATATAAAAATAAAAGTGTCTGATGTAaaaaaaaaaaaaaaaaaaaaACTCG
 polyadenylation signal poly (A) tail (26)

537 AGAGTATTCTAGAGCGGCCGCGGCCATCGTTTCCACCC

Fig. 2.6a

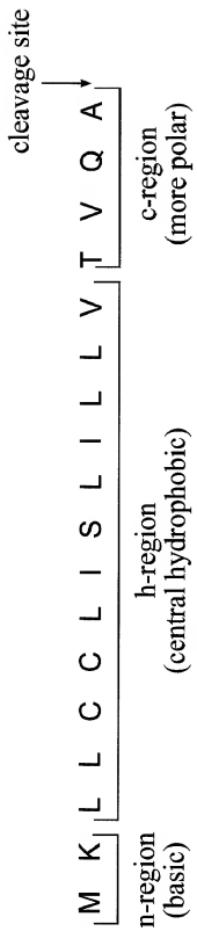


Fig. 2.66

A. Mature Tm 13.17 amino acid residue

1 LTEAQIEKLN KISKKCQNES GVSQEIIITKA RNGDWEDDPK LKRQVFCVAR
51 NAGLATESGE VVVDVLREKV RKVTDNDEET EKIINKCAVK RDTVEETVFN
101 TFKCVMKNKP KFSPVD

B. Summary of the composition analysis for the mature Tm 13.17 sequence:

<u>Residue</u>	<u>Number</u>	<u>Mole Percent</u>
A = Ala	6	5.172
B = Asx	0	0.000
C = Cys	4	3.448
D = Asp	8	6.897
E = Glu	13	11.207
F = Phe	4	3.448
G = Gly	4	3.448
H = His	0	0.000
I = Ile	6	5.172
K = Lys	16	13.793
L = Leu	5	4.310
M = Met	1	0.862
N = Asn	8	6.897
P = Pro	3	2.586
Q = Gln	4	3.448
R = Arg	6	5.172
S = Ser	5	4.310
T = Thr	8	6.897
V = Val	14	12.069
W = Trp	1	0.862
Y = Tyr	0	0.000
Z = Glx	0	0.000

Molecular weight = 13171.96; Residues = 116; Average Residue Weight = 113.551

Charge = 1; Isoelectric point = 7.74.

Fig. 2.6c

1	A G T G G A T C C A A R G A T T C O O C A C G A G A C T A C T A G A T G A A	Tm 13.17 B 1
41	G T T O C T C T O T T O T C T R A T C I C C C T C A T T C T O T T O G T C R A C A	Tm 13.17 B 1
1		
81	G T T C A G G C C C T T O A C C G A G G O C C A C A A T T G A O A R A C T O R A C A	Tm 13.17 B 1
28	G T T C A G G C C A T A R A C T C A G G A A G A C C T T G A O C T A C T O C C C C C	Tm 13.17 B 1
121	A G A T C A G C A A R A R A T O T C T C A A R A T T G A O A R A C T O R A C A	Tm 13.17 B 1
68	A A A C C C R O C O C C A A G O T G C A A B O A C T O R A C T O R A C T O G T C C O A	Tm 13.17 B 1
161	A G A T C A T A R A C C C A A R G C T C O C A R C O O T O A C T T G G G G A C	Tm 13.17 B 1
118	A G A T C O T C A T A R A O A G A G C T C O C A R A C O O T O A C T T G G G G A C	Tm 13.17 B 1
201	G A T C C T A A R A C T O R A R A C C C A R A O T T T T T G C O T G G G C C R O O A	Tm 13.17 B 1
148	G R C C C C C T A A R A C T O R A R A T O C A R C T T T T T O C A R A T T T C A R D O	Tm 13.17 B 1
241	A C O C C C O T C T G O C C A C A G O R A T C O G G G G A G G O T O G T G G T C O A	Tm 13.17 B 1
188	C R C T C O R A R A Y T O T C O C C O R A T C O O D C A R A A T T O A O O C C O A	Tm 13.17 B 1
281	C O T O T T O T O O O O A R A O A O G T O A O O A R A O O T C A C T O C A C A C G C	Tm 13.17 B 1
228	C R C O T T C A R O G R O A R O T T O A C G A A G T O A C R A C R A C A G T O A T	Tm 13.17 B 1
321	G A G A R A C T O D A G A R A R A T C T C A T T A R G O T C O C C O T C C R A O A	Tm 13.17 B 1
268	G A G A R A R A D C O R A R G T T G T C O R G A R G T O C A C O O T O R A C T C	Tm 13.17 B 1
361	G A G A T A C T O T T G A R A D O A C O G T O T T C A R T A T C T T C A R A T O	Tm 13.17 B 1
308	A A C A C R A C T C C O D A R A T A C C O C C A T T T O A R O T T A C C C A R A T C	Tm 13.17 B 1
401	T O T C A T O A A A A C A R O C C A A R O T T C T C A C C A G T T G T A T T T O A	Tm 13.17 B 1
348	T O T T A T T O R A O O A C A R G C C C A T T T T C T	Tm 13.17 B 1
441	A C C A C C A C G A C T T G A T O G T T C T C A R A T O G T G T T O C T T T T A C	Tm 13.17 B 1
388	A O A C T A T T T O T C T G A A A	Tm 13.17 B 1
481	A T T A T T A A R A R A T T A R A G T G T T T C T O R T O T A A A A A A A A A A A A	Tm 13.17 B 1
428	O C A C C A A	Tm 13.17 B 1

Fig. 2.7

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Percent identity: 39.8 (identical amino acids; Percent similarity: 58.3 (identical amino acids plus conservative amino acids).

Fig. 2.8

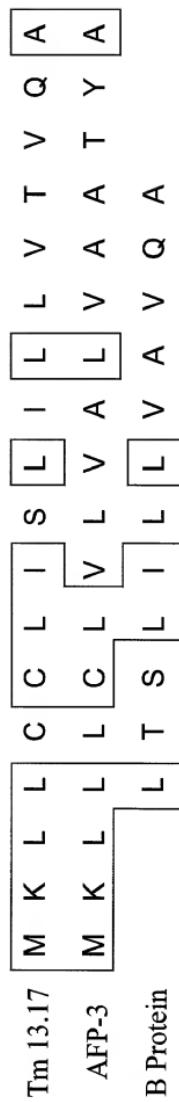


Fig. 2.9

Tm 13.17 NH2 [L T] E A [Q I] E [K] L [N K I S K] K C [Q] N E
Tm 12.86 NH2 [L T] D E [Q I] Q [K] R [N K I S K] E ? [Q] Q V

Fig 2.10

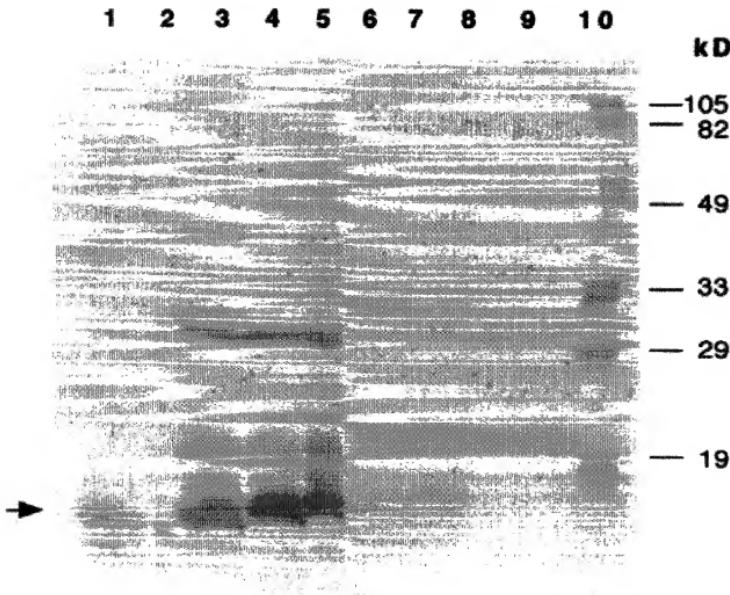


Fig 2.11

Tm 12.86	L T D E Q I Q K R N K I S K E ? Q Q V
Tm 13.17 1	L T E A Q I E K L N K I S K K C Q N E S G V S Q E I I T K A
B1 13	I T E E D L E L L R Q T S A E C K T E S G V S E D V I K R A
APP-3 1	E T P R E K L K Q H S D A C K A E S G V S E E S L N K V
Tm13.17 31	R N G D W E D D P K L K R Q V F C V A R N A G L A T E S G E
B1 44	R K G D L E D D P K L K M Q L L C I F K A L E I V A E S G E
APP-3 29	R N R E E V D D P K L K E H A F C I L K R A G F I D A S G E
Tm13.17 61	V V V D V L R E K V R K V T D N D E E T E K I I N K C A V K
B1 75	I E A D T F K E K L T R V T N D D E E S E K I V E K C T V T
APP-3 59	F Q L D H I K T K F K E N S E H P E K V D D L V A K C A V K
Tm13.17 91	R D T V E E T V F N T F K C V M K N K P K F S P V D
B1 106	E D T P E D T A F E V T K C V L K D K P N F F G D L F V
APP-3 89	K D T P Q H S S A D F F K C V E D N R S

Fig. 2.12

200726 064348ED

1 GGCACGAGCAAAATGAAACTCCCTCTGTGCTTGC~~G~~T~~C~~GGCC
 M K L L L C F A F A A

47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATA~~C~~AGAAA
 I V I G A Q A L T D E Q I Q K

92 AGGAACAAGATCAGCAAAGATGCCAGCAGGTGTCCGGAGTGTCC
 R N K I S K E C Q Q V S .G V S

137 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTGGTCGATGAT
 Q E T I D K V R T G V L V D D

182 CCCAAAATGAAGAACGACGTCCCTCTGCTTCTCGAAAGAAAACTGGA
 P K M K K H V L C F S K K T G

226 GTGGCAACCGAAGGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
 V A T E A G D T N V E V L K A

271 AAGCTGAAGCATGTGGCCAGCGACGAAGAGGGTGACAAAGATCGT G
 K L K H V A S D E E V D K I V

316 CAGAAGTGC~~G~~TGGTCAAGAAGGCCACACCCAGAGGAAACGGCTTAT
 Q K C V V K K A T P E E T A Y

361 GACACCTTCAAGTGTATTTACGACAGCAACCTGATTTCTCTCCT
 D T F K C I Y D S K P D F S P

406 ATTGATTAATTGTTTGATTTGACTGAATTTGACAATAAAGGT
 I D

polyadenylation signal

451 ACTATCGTTATGTAAAAAAAAAAAAAA

poly (A) tail

Fig. 3.0

1 GGCACGAGCAAAATGAAACTCCTCTTGTGCTTGCTTCGCCGCC
 M K L L C F A F A A

47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATAACAGAAA
 I V I G A Q A L T D E Q I Q K ↑

92 AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCC
 R N K I S K E C Q Q V S G V S

137 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
 Q E T I D K V R T G V L V D D

182 CCCAAATGAAGAAGCACGTCCTCTGCTTCTCGAAAGAAAACGGAA
 P K M K K H V L C F S K K T G

226 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
 V A T E A G D T N V E V L K A

271 AAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTG
 K L K H V A S D E E V D K I V

316 CAGAAGTGCCTGGTCAAGAACGGCCACACCAAGAGGAAACGGCTTAT
 Q K C V V K K A T P E E T A Y

361 GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCT
 D T F K C I Y D S K P D F S P

406 ATTGATTAATTGTTTGTATTTGACTGAATTGACAAAAGGT
 I D .
 polyadenylation signal

451 ACTATCGTTATGAAAAAAAAAAAAAA

poly (A) tail

Start

↓

2-2	G G C A C G A G C A A A A A T G A A A C T C C T C T T G T G C T T T G C	G
2-3	G G C A C G A G C A A A A A T G A A A C T C C T C T T G T G C T T T G C	T
2-2	T T C G C C G C C A T C G T C A T C G G A G C T C A G G C T C T C A C C G	
2-3	T T C G C C G C C A T C G T C A T C G G A G C T C A G G C T C T C A C C G	
2-2	A C G A A C A G A T A C A G A A A A G G A A C A A G A T C A G C A A A G A	
2-3	A C G A A C A G A T A C A G A A A A G G A A C A A G A T C A G C A A A G A	
2-2	A T G C C A G C A G G T G T C C G G A G T G T C C C A A G A G A C G A T C	
2-3	A T G C C A G C A G G T G T C C G G A G T G T C C C A A G A G A C G A T C	
2-2	G A C A A A G T C C G C A C A G G T G T C T T G G T C G A T G A T C C C A	
2-3	G A C A A A G T C C G C A C A G G T G T C T T G G T C G A C G A T C C C A	
2-2	A A A T G A A G A A G C A C G T C C T C T G C T T C T C G A A G A A A A C	
2-3	A A A T G A A G A A G C A C G T C C T C T G C T T C T C G A A G A A A A C	
2-2	T G G A G T G G C A A C C G A A G G C C G G A G A C A C C A A T G T G G A G	
2-3	T G G A G T G G C A A C C G A A G G C C G G A G A C A C C A A T G T G G A G	
2-2	G T A C T C A A A G C C A A G C T G A A G C A T G T G G C C A G C G A C G	
2-3	G T A C T C A A A G C C A A G C T G A A G C A T G T G G C C A G C G A C G	
2-2	A A G A [G] T G G A C A A G A T C G T G C A G A A G T G C G T G G T C A A	
2-3	A A G A [A] T G G A C A A G A T C G T G C A G A A G T G C G T G G T C A A	
2-2	G A A G G C C A C A C C A G A G G A A A C G G C T T T A T G A C A C C T T C	
2-3	G A A G G C C A C A C C A G A G G A A A C G G C T T T A T G A C A C C T T C	
2-2	A A G T G T A T T T A C G A C A G [C] A A A C C T G A T T T C T C T C C T A	
2-3	A A G T G T A T T T A C G A C A G [T] A A A C C T G A T T T C T C T C C T A	
2-2	T T G A T T A A T T G T T T G T A T T T G A C T G A A T T T T G A C A A	
2-3	T T G A T T A A T T G T T T G T A T T T G A C T G A A T T T T G A C A A	
2-2	T A A A G G T A [A] T A T C G T T A T G [T] A A A A A	
2-3	T A A A G G T A [C] T A T C G T T A T G [A] A A A A A	

Fig 3.2

Predicted Amino Acid

Composition of 2-2 and 2-3

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
V Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
Ter	0	0.00	0.00

Fig. 3.3

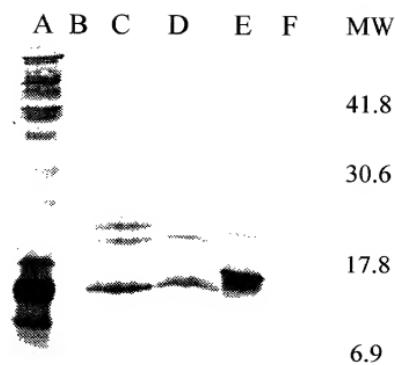


Fig. 3.4

200875795 - 621200

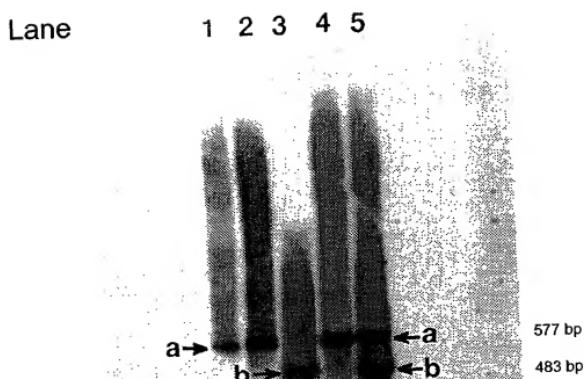


Fig. 4.0

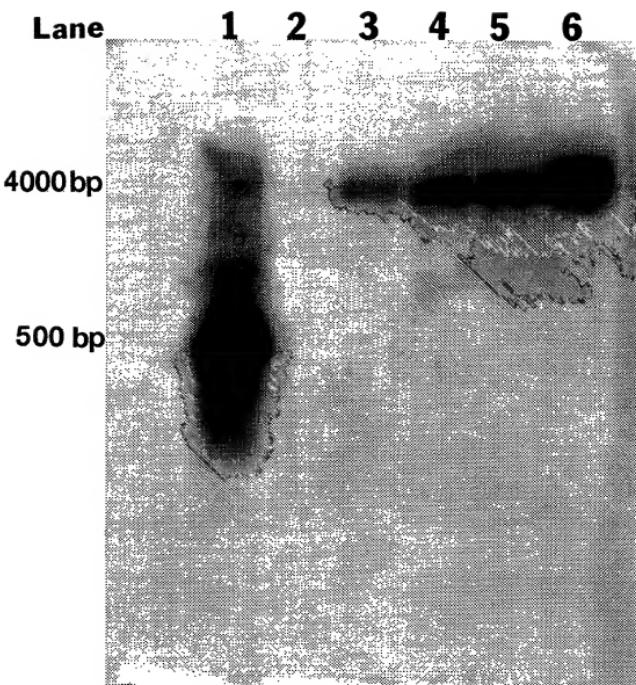


Fig. 4.1

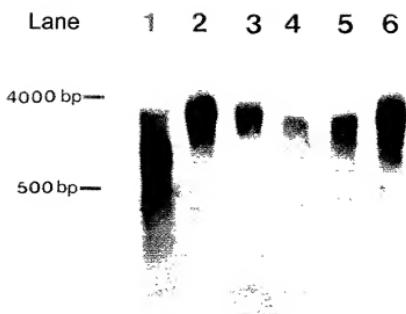
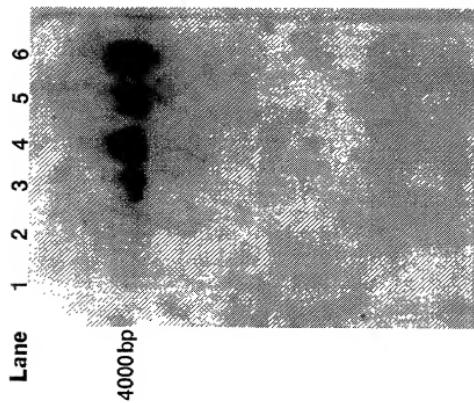


Fig. 4.2

A.



B.

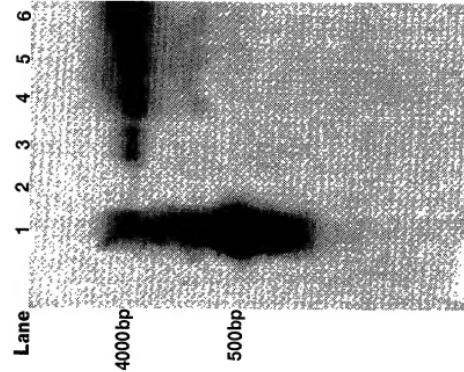


Fig. 4.3

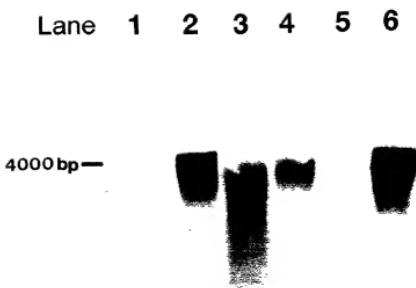
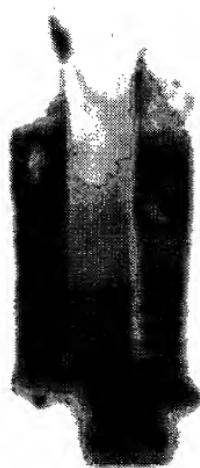


Fig. 4.4

Lane 1 2 3 4 5



23130

9416

4361

2322

2027

564

Fig. 4.5.

Tm 13.17 cDNA

1 AGTGGATCCARAGAATT CGGCACGAGACTACTAAG ~~ATG~~AGTTGCTCTGTTGTCTAATCT
M K L L C C L I S

61 CCCTCATTCTGTTGGTCACAGTCAGGCCCTGACCGAGGCACAAATTGAGAAACTGAACA
L I L L V T V Q A L T E A Q I E K L N K
Forward Primer

121 AGATCAGCAAAAATGTCAAAATGAAAGTGGAGTGTGCGCAAGAGATCATAACCAAAGCTC
I S K K C Q N E S G V S Q E I I T K A R

181 GCAACGGTGA C T G G G A G G A C G A T C C T A A A C T G A A A C G C C A A G T T T T G C G T G G C C A G G A
N G D W E D D P K L K R Q V F C V A R N

241 A C G C C G G T C T G G C C A C G G A A T C G G G A G A G G T G G T G G T C G A C G T G T T G A G G G A A A G G T G A
A G L A T E S G E V V V D V L R E K V R

301 G G A A G G T C A C T G A C A A C G A C G A A G A A C T G A G A A A T C A T C A A T A A G T G C G C C G T C A A G A
K V T D N D E E T E K I I N K C A V K R
Reverse Primer

361 G A G A T A C T G T G A A G A G A C G G T G T C A A T C T T C A A T G T G T C A T G A A A A C A A G C C A A
D T V E E T V F N T F K C V M K N K P K

421 A G T T C T C A C C A G T T G A T T G A A C C A C C A C G A C T A G T A G A T G G T T C A A A T G G T G T G C T T A C
F S P V D *

481 A T A A A A A T A A A G T G T T C T G A T G T A C T C G
polyadenylation signal poly (A) tail (26)

537 A G A G T A T T C T A G A G C G G C C C G G C C C A T C G T T T C C A C C C

Fig. 4.6a

Forward Primer

2-2	L T D E Q I Q K R N K I S K E C Q Q V S [G V S Q E T I D K V R T G V L V
Tm 13.17	L T E A O I E K L N K I S K K C Q N E S [G V S Q E I I T K A R N G D W E
B2	L T E E D L Q L L R Q T S A E C K T E S [G A S E A V I K K A R K G D L E
AFP-3	E T P R E K L K Q H S D A C K A E S [G V S E E S L N K V R N R E E V
2-2	D D P K M K K H V L C F S K K T G V A T E A G D T N V E V L K A K L K H
Tm 13.17	D D P K L K R Q V F C V A R N A G L A T E S G E V V V D V L R E K V R K
B2	D D P K L K M Q L L C I F K A L E I V A E S G E I E A D T F K E K L T R
AFP-3	D D P K L K E H A F C I L K R A G F I D A S G E F Q L D H I K T K F K E

Reverse Primer

2-2	V A S D E E V D K I V Q K C V V K K [A T P E E T A Y D T F K C I Y D S
Tm 13.17	V T D N D E E T E K I I N K C A V K R I D T V E E T V F N T F K C V M K N
B2	V T N D D E E S E K I V E K C T V T E D T P E D T I A F E V T K C V L K D
AFP-3	N S E H P E K V D D L V A K C A V K K [D T P O H S I S A D F F K C V H D N
2-2	K P D F S P I D
Tm 13.17	K P K F S P V D
B2	K P N F F G D L F V
AFP-3	R S

Fig. 4.6b

Primer	percent % composition				Melting Temperature (°C)
	A	C	G	T	
Forward	28.6	14.3	42.9	14.3	44.0
Reverse	25.0	31.3	6.3	37.5	44.0

Fig. 4.6c

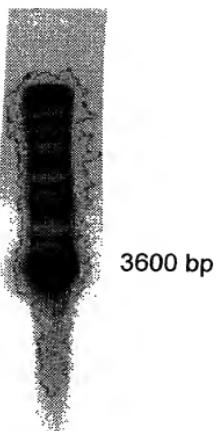


Fig. 4.7

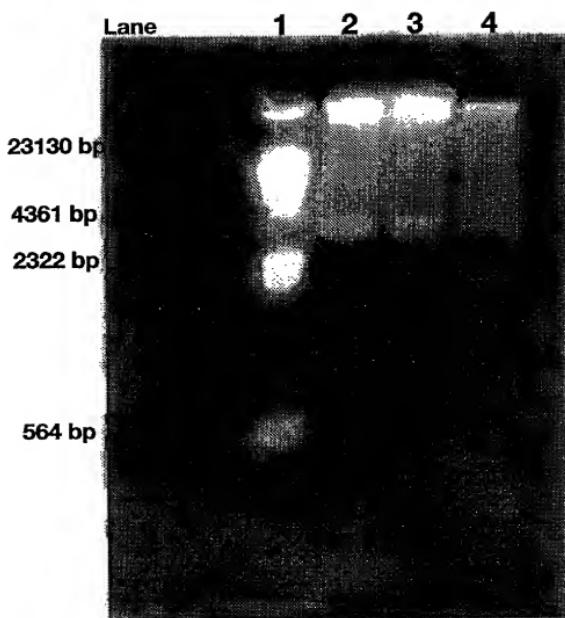


Fig. 4.8

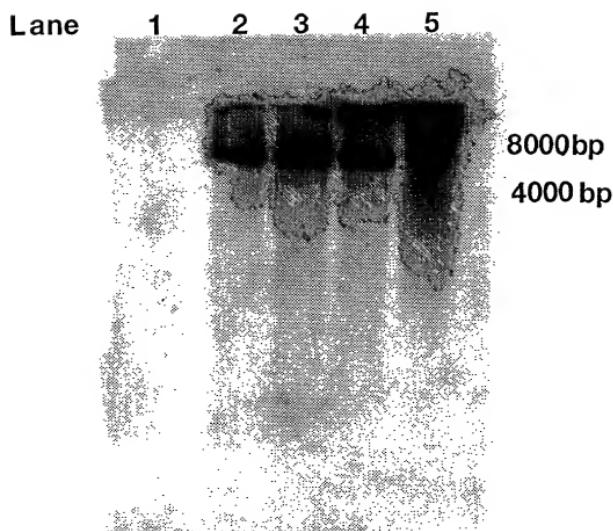


Fig. 4.9

卷之三

1 GGCACGAGCAAAATGAAACTCTCTTGTGCTTGTCTTCGCCGCC
 M K L L L C F A F A A

47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATAACAGAAA
 I V I G A Q A L T D E Q I Q K

92 AGGAACAAGATCAGCAAAAGAATGCCAGCAGGGTGTCCGGAGTGTCC
 R N K I S K E C Q Q V S G V S

137 CAAGAGAGCAGATCGACAAAGTCCGCGACAGGGTGTCTTGGTCGACGAT
 Q E T I D K V R T G V L V D D

182 CCCAAATGAAGAAGCAGCTCTCTGCTTCTCGAAGAAACTGGAA
 P K M K K H V L C F S K K T G

226 GTGGCAACCGAAGGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
 V A T E A G D T N V E V L K A

271 AAGCTGAAGCATGTCGCCAGCAGAAGAGGGTGGACAAGATCGTG
 K L K H V A S D E E V D K I V

315 CAGAAGTGCCTGGTCAAGAGGCCACACCCAGAGGAAACCGCTTAT
 Q K C V V K K A T P E E T A Y

361 GACACCTTCAAGGTTATTCAGCAGCTAAACCTGATTTCCTCTCCT
 D T F K V I Y D S K P D F S P

406 ATTGATTAATTGTTTGTATTGACTGAATTGGACAAAAAAGGT
 I D *

451 ACTATCGTTATGTAAAAAAAAAAAAAAAAAA polyadenylation signal

poly (A) tail

Fig. 4.10a

Predicted Amino Acid

Composition of 3-4

Molecular Weight	12839.70 m.w.
Length	115
1 microgram =	77.883 pMoles
Molar Extinction coefficient	2920±5%
1 A(280) =	4.40 mg/ml
Isoelectric Point	7.14
Charge at pH 7	0.16

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	47	46.41	40.87
Acidic (DE)	20	18.91	17.39
Basic (KR)	20	20.41	17.39
Polar (NCQSTY)	29	24.55	25.22
Hydrophobic (AIFWV)	35	28.04	30.43
A Ala	6	3.32	5.22
C Cys	3	2.41	2.61
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.99	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.09	7.83
V Val	15	11.58	13.04
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
Ter	0	0.00	0.00

Fig. 4.10b

1 GGCACGAGCAAAAATGAAACTCCCTTGTGCTTGCTTCGCCGCC
 M K L L L C F A F A A

47 ATCGTCATCGGAGCTCAGGCTCTCACCGATGAACAGATACAGAAA
 I V I G A Q A L T D E Q I Q K ↑

92 AGGAACAAAGATCAGCAAAGAATGCCAGCAGGAGTCCGGAGTGTCC
 R N K I S K E C Q Q E S G V S

137 CAAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
 Q E T I D K V R T G V L V D D

182 CCCAAAAATGAAGAAGCAGCTCCTCTGCTTCTCGAAAGAGAACTGGA
 P K M K K H V L C F S K R T G

226 GTGGCAACCGAACGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
 V A T E A G D T N V E V L K A

271 AAGCTGAAGCATGTGGCCAGCGACGAAAGAAGTGGACAAAGATCGT G
 K L K H V A S D E E V D K I V

316 CAGAAGTGCCTGGTCAAGAACGCCACACCAAGAGAAACGGCTTAT
 Q K C V V K K A T P E E T A Y

361 GACACCTTCAAGTGTATTACGACAGTAAACCTGATTTCTCTCCT
 D T F K V I Y D S K P D F S P

406 ATTGATTAATTGTTGTATTTGACTGAATTTGACAATAAAAGGT
 I D polyadenylation signal

451 ACTATCGTTATGAAAAA
 poly (A) tail

Fig. 4.11a

Predicted Amino Acid

Composition of 3-9

Analysis	Whole Protein
Molecular Weight	12871.80 m.w.
Length	115
1 microgram =	77.689 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.23 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.31	41.74
Acidic (DE)	20	18.86	17.39
Basic (KR)	20	20.57	17.39
Polar (NCQSTY)	30	25.29	26.09
Hydrophobic (AILFWV)	34	27.20	29.57
A Ala	6	3.31	5.22
C Cys	4	3.21	3.48
D Asp	11	9.84	9.57
E Glu	9	9.03	7.83
F Phe	3	3.43	2.61
G Gly	4	1.77	3.48
H His	2	2.13	1.74
I Ile	6	5.28	5.22
K Lys	17	16.93	14.78
L Leu	5	4.40	4.35
M Met	1	1.02	0.87
N Asn	2	1.77	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.97	5.22
R Arg	3	3.64	2.61
S Ser	7	4.74	6.09
T Thr	9	7.07	7.83
V Val	14	10.78	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
Ter	0	0.00	0.00

Fig. 4.11b

1 GGCACGAGCAAAA ATGAAACTCCTCTTGTGCTTTCGCCGCC
 M K L L L C F A F A A

47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATAACAGAAA
 L V I G A Q A L T D E Q I Q K ↑

92 AGGAACAAAGATCAGCAAAAGAGTGCCAGCAGGTGTCCGGAGTGTCC
 R N K I S K E C Q Q E S G V S

137 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTGGTCGACGAT
 Q E T I D K V R T G V L V D D

182 CCCAAATGAAGAACGACGTCTCTGCTTCTCGAAAGAAACTGGAA
 P K M K K H V L C F S K R T G

226 GTGGCAACCGAACGGAGACACCAATGTGGAGGTACTCAAAGGCC
 V A T E A G D T N V E V L K A

271 AAGCTGAAGCATGTGGCCAGCAGAAGAAGTGGACAAAGATCGTG
 K L K H V A S D E E V D K I V

316 CAGAAGTGCCTGGTCAAGAAGGCCACACCAAGGGAAACGGCTTAT
 Q K C V V K K A T P E E T A Y

361 GACACCTTCAAGTGTATTACGACAGTAAACCTGATTTCTCCCT
 D T F K V I Y D S K P D F S P

406 ATTGATTAATTGTTTGTATTTGGCTGAATTTGACAAAATAAGGT
 I D ↑ polyadenylation signal

451 ACTATCGTTATGTAAAAAAAAAAAAAA

poly (A) tail

Fig. 4.12a

Predicted Amino Acid

Composition of 7-5

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AIFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
V Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

Fig. 4.12b

202320-95294860

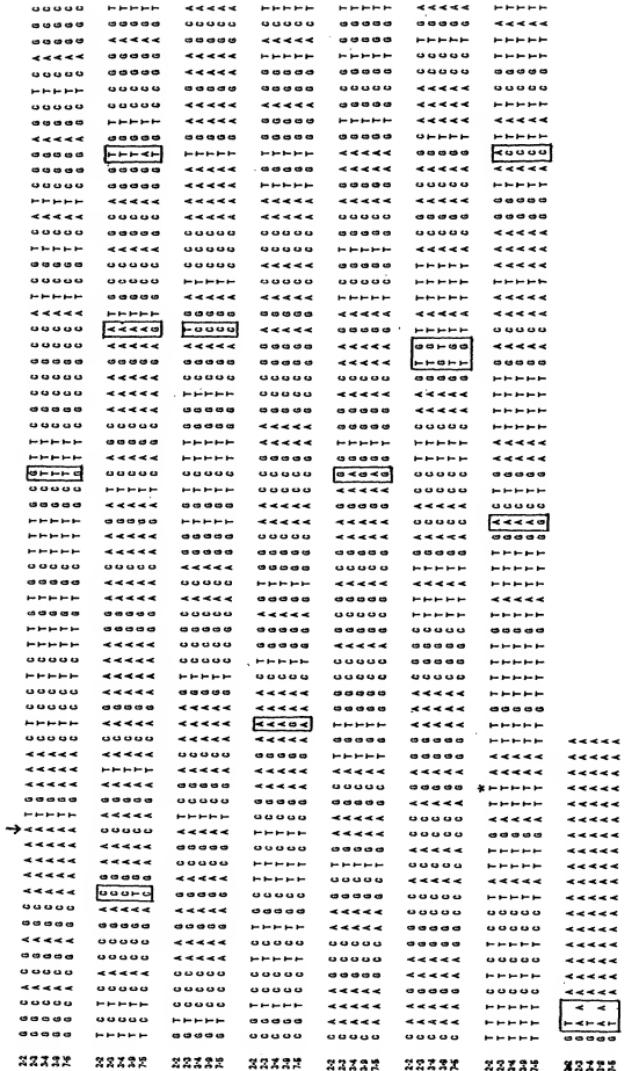


Fig 4.13

2-2	M K L L L C F A F A A V G A Q A L T D E Q Q K R N K S K E C Q Q V S S Q E T D K V R T G V L V	D D P K M K K K H V L C F S K K T G V A T E A G D T N V E V L K A K L K H V A S D E E V D K I V Q K C V V K K	A T P E E T A Y D T F K C Y D S K P D F S P D *	2-2
2-3	M K L L L C F A F A A V G A Q A L T D E Q Q K R N K S K E C Q Q V S S Q E T D K V R T G V L V	D D P K M K K K H V L C F S K K T G V A T E A G D T N V E V L K A K L K H V A S D E E V D K I V Q K C V V K K	A T P E E T A Y D T F K C Y D S K P D F S P D *	2-3
3-4	M K L L L C F A F A A V G A Q A L T D E Q Q K R N K S K E C Q Q V S S Q E T D K V R T G V L V	D D P K M K K K H V L C F S K K T G V A T E A G D T N V E V L K A K L K H V A S D E E V D K I V Q K C V V K K	A T P E E T A Y D T F K C Y D S K P D F S P D *	3-4
3-9	M K L L L C F A F A A V G A Q A L T D E Q Q K R N K S K E C Q Q V S S Q E T D K V R T G V L V	D D P K M K K K H V L C F S K K T G V A T E A G D T N V E V L K A K L K H V A S D E E V D K I V Q K C V V K K	A T P E E T A Y D T F K C Y D S K P D F S P D *	3-9
7-5	M K L L L C F A F A A V G A Q A L T D E Q Q K R N K S K E C Q Q V S S Q E T D K V R T G V L V	D D P K M K K K H V L C F S K K T G V A T E A G D T N V E V L K A K L K H V A S D E E V D K I V Q K C V V K K	A T P E E T A Y D T F K C Y D S K P D F S P D *	7-5

Fig. 4.14

MW (kDa)	AA (#)	(% mole)										Trp	Ass	Glx	Arg	Lys	Ser	% most hydrophilic				
		Cys	Pro	Phe	Ile	Val	Met	Leu	% most hydrophobic	Gly	Ala	Tyr	His									
Tm 12.86	117	3.2	3.0	3.4	4.4	8.5	2.0	4.4	28.9	3.1	3.9	3.8	3.2	ND	10.7	15.0	3.6	14.9	6.8	6.3	57.3	
Tm 13.17	116	3.13	2.21	4.47	5.16	10.5	1.0	4.3	29.11	1.73	3.24	0	0	1.41	0	0	7.12	15.6	3.31	6.14	32.14	
2.2	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	2.43	18.0	4.75	7.08	32.23	
2.3	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.08	32.23
3.4	12.84	115	2.41	3.02	3.44	5.29	11.6	1.02	4.41	28.04	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.09	32.24
3.9	12.87	115	3.21	3.02	3.43	5.28	10.8	1.02	4.40	27.20	1.77	3.31	2.54	2.13	0	0	0	3.64	16.9	4.74	7.07	32.38
7.5	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	2.43	18.0	4.75	7.08	32.23	

Fig. 4.15

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1

Tm 12.88
2-2
2-3
3-4
3-8
7-5
Tm 13.17
B1
E2
AFP.3

2-2
2-3
3-4
3-9
7-5
Tm 13.1
B1
B2
AFP-3

2-2
2-3
3-4
3-9
7-5
Tm 13.17
B1
B2
APP-3

FIG 4.16 α -concentrated cysteine (yellow)

Boxed = conserved residues in >7 Genes (blue or orange)

Fig. 4.16

Tm 13.17	22	B1 prot	L T E Q T T E K C N K I S	K K T Q N E S S V S Q E T T I K A	R N G D	34
		B2 prot	L T E E D L E K C N K I S	K E G C T T E S S V S Q E T T I K A	R K G D	34
		Pip_Antico	L T E E D L E K C N K I S	K E G C T T E S S V S Q E T T I K A	R K G D	34
		Pip_Mausa	S P Q V M K N L S U N F G K A M D Q C K D E I S L P D S V U V A D Y N F W K D	R K G D	34	
		Obp2_Mausa	D V Q V M K D V T L G F G O A L E Q C R E E S O L P E S L P E M D E F K H F W R E D	R K G D	40	
		Pipop2_Droma	Q E P R D G E W P P A I T K G K F H	D I C A P K T G V T D E A I K F F	S D G Q	40
		Phop2_Droma	Q R D E N Y P P P G I L K Q V R Y R	D A C V E F K F M R C L M Q T A S V D V D Q S	S D G Q	40
		Popop2_Droma	E I N P T I K N D H L L E L A	N E C K R E T G A T D E O V T L	V K N A	35
		Popop2_Droma			M S H H	36
Boxed = conserved residues in Genes (red)						
Tm 13.17	22	B1 prot	W E D D P K Y R Q V F V A T I N A S G A T E S G E V V D V T R E X T R K V T D N D E E T E I	S P V D	81	
		B2 prot	Y E D D P K L K M Q I L C F K A L E V A E S G E I E A D T F K E X L T R V T N D E E S E	S P V D	80	
		Pip_Antico	Y E M T D R I A G C A I N C A T T Y D V D D G N L H G N A K D F A M K R H G A D E T M A Q Q L	S E E S E	81	
		Pip_Mausa	F K F E H R E T G C A T T C M S R R H F N L D P D E C K L H G N A M E F A K K H G A D E A M A Q Q L	S E E S E	90	
		Obp2_Mausa	F E D V V H R E L G C A T T C M S N K F E L L O D D T R H Y H V K M H D Y I K S E P P A G Q U V S E K M	S E E S E	90	
		Phop2_Droma	I H E D E K L K C Y M N C F E H E I E V Y V D O N G T Y H M E V L N A P G E K L R N I M M E	S E E S E	90	
		Popop2_Droma	I H E D E K L K C Y M N C F E H E I E V Y V D O N G T Y H M E V L N A P G E K L R N I M M E	S E E S E	90	
		Popop2_Droma	I C P T D P E T K C F Y C C M F D M F G I D S O N I M H L E A L E V Y V P E E I Y K T I N S	S E E S E	86	
		Popop2_Droma	I C P T D P E T K C F Y C C M F D M F G I D S O N I M H L E A L E V Y V P E E I Y K T I N S	S E E S E	82	
* - conserved cysteine (yellow)						
Tm 13.17	22	B1 prot	K I V Y G C T V Y K	R P Q T V E E T V F L N T F R C G M M N K X F	S P V D	116
		B2 prot	K I V E K C T V T E D	E T A D T F K C Y D S K P D F F S P I D	S P V D	115
		Pip_Antico	V D I H G C E K S A P N D O K C K Y T D T A F E V T K C V L K D K P N F E	F G D L F V	118	
		Pip_Mausa	V D I H G C E N S T A P P N D C K Y T D T A F E V T K C V L K D K P N F E	F G D L F V	118	
		Obp2_Mausa	V O T I H N C E E K Q Y D D I A D D C D R K H K W Q H Q C W K K D P X H Y F L P	E V A M I E V	137	
		Phop2_Droma	A S K G C V H P E G D T L C H K A W F H Q C W K K D P X H Y F L P	E V A M I E V	125	
		Popop2_Droma	L V S S C G T O K S D G C D T A V E V C G I V A N G K F I W E E I V L L G	E V A M I E V	121	
		Popop2_Droma	P A E V V A R C E A I E T P E D H C A T M A E L A V E C G I V A N G K F I W E E I V L L G	E V A M I E V	123	
		Popop2_Droma			E Q M R E H G L E L E H	127

Fig. 4.77

Tm 12.88

22	M	X	L	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	V
23	M	K	L	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	V
34	M	K	L	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	V
39	M	K	L	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	V
75	M	K	L	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	V
Tm 13.17	M	K	L	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	V
B1	M	X	L	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	V
B2	M	X	L	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	V
AF3	M	X	L	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	V

22

22	D	P	K	M	X	H	V	L	C	F	S	K	K	T	G	V	A	T	E	A	G	D	T	N	V	E	V	L	K	A	K	L	K	H	V	A	S					
23	D	P	K	M	K	K	H	V	L	C	F	S	K	K	T	G	V	A	T	E	A	G	D	T	N	V	E	V	L	K	A	K	L	K	H	V	A	S				
34	D	P	K	M	K	K	H	V	L	C	F	S	K	K	T	G	V	A	T	E	A	G	D	T	N	V	E	V	L	K	A	K	L	K	H	V	A	S				
39	D	P	K	M	K	K	H	V	L	C	F	S	K	K	T	G	V	A	T	E	A	G	D	T	N	V	E	V	L	K	A	K	L	K	H	V	A	S				
75	D	P	K	M	K	K	H	V	L	C	F	S	K	K	T	G	V	A	T	E	A	G	D	T	N	V	E	V	L	K	A	K	L	K	H	V	A	S				
Tm 13.17	D	P	K	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	S
B1	D	P	K	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	S
B2	D	P	K	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	S
AF3	D	P	K	L	C	F	A	A	V	I	G	A	Q	A	L	T	D	Q	I	Q	K	N	K	I	S	K	E	G	V	S	Q	E	T	I	D	K	V	R	T	G	V	S

22

22	A	T	P	E	E	T	A	Y	D	T	F	K	C	I	Y	D	S	K	P	D	F	S	P	I	D												
23	A	T	P	E	E	T	A	Y	D	T	F	K	C	I	Y	D	S	K	P	D	F	S	P	I	D												
34	A	T	P	E	E	T	A	Y	D	T	F	K	C	I	Y	D	S	K	P	D	F	S	P	I	D												
39	A	T	P	E	E	T	A	Y	D	T	F	K	C	I	Y	D	S	K	P	D	F	S	P	I	D												
75	A	T	P	E	E	T	A	Y	D	T	F	K	C	I	Y	D	S	K	P	D	F	S	P	I	D												
Tm 13.17	D	T	P	E	D	T	A	E	V	T	K	C	V	M	N	K	P	K	P	V	D	L	F	V													
B1	D	T	P	E	D	T	A	E	V	T	K	C	V	M	N	K	P	K	P	V	D	L	F	V													
B2	D	T	P	E	D	T	A	E	V	T	K	C	V	M	N	K	P	K	P	V	D	L	F	V													
AF3	D	T	P	E	D	T	A	E	V	T	K	C	V	M	N	K	P	K	P	V	D	L	F	V													

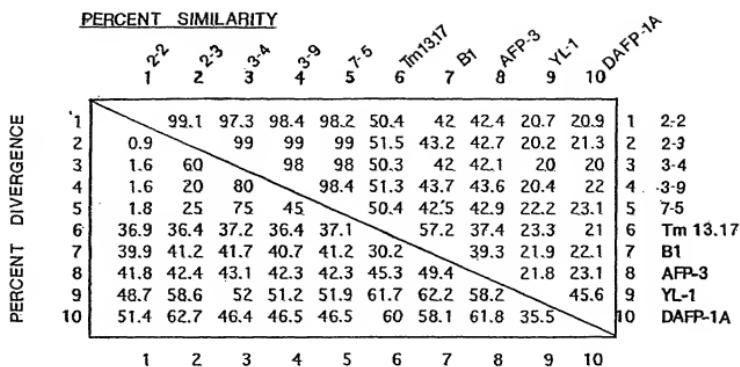
FIG 4.18

* = conserved cysteine (yellow)

Boxed = conserved residues in Genes (red, green, orange or blue)

Fig. 4.18

NUCLEOTIDE SEQUENCES



AMINO ACID SEQUENCES

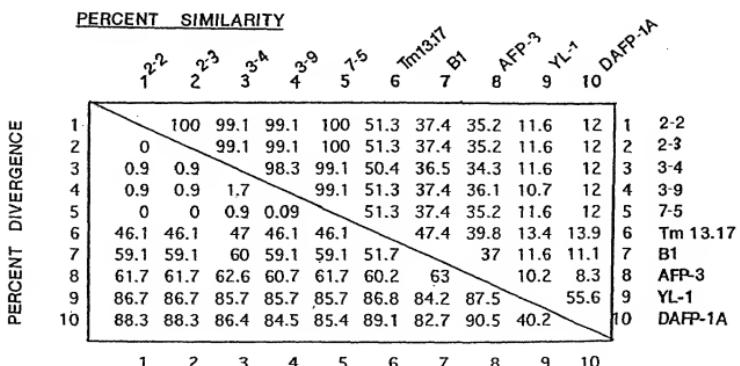


Fig. 4.19

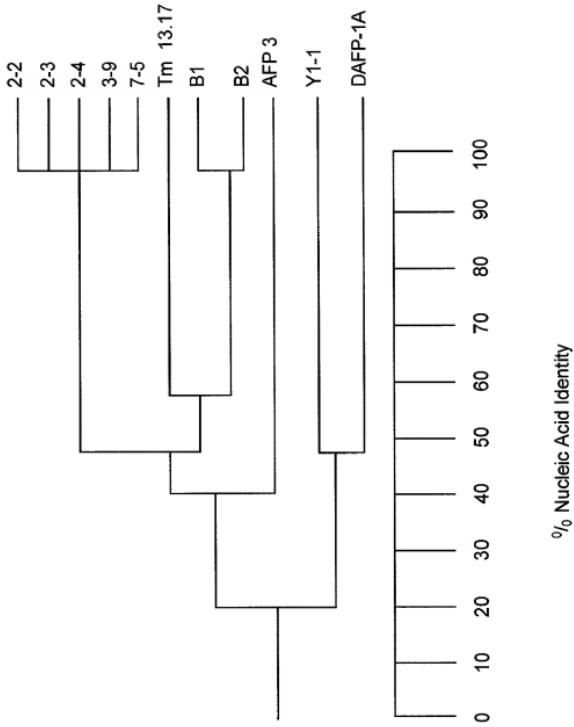


Fig. 4.20

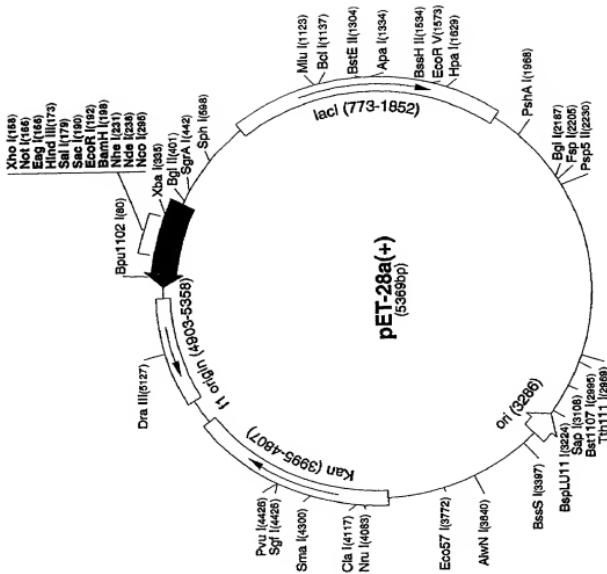


Fig. 5.0

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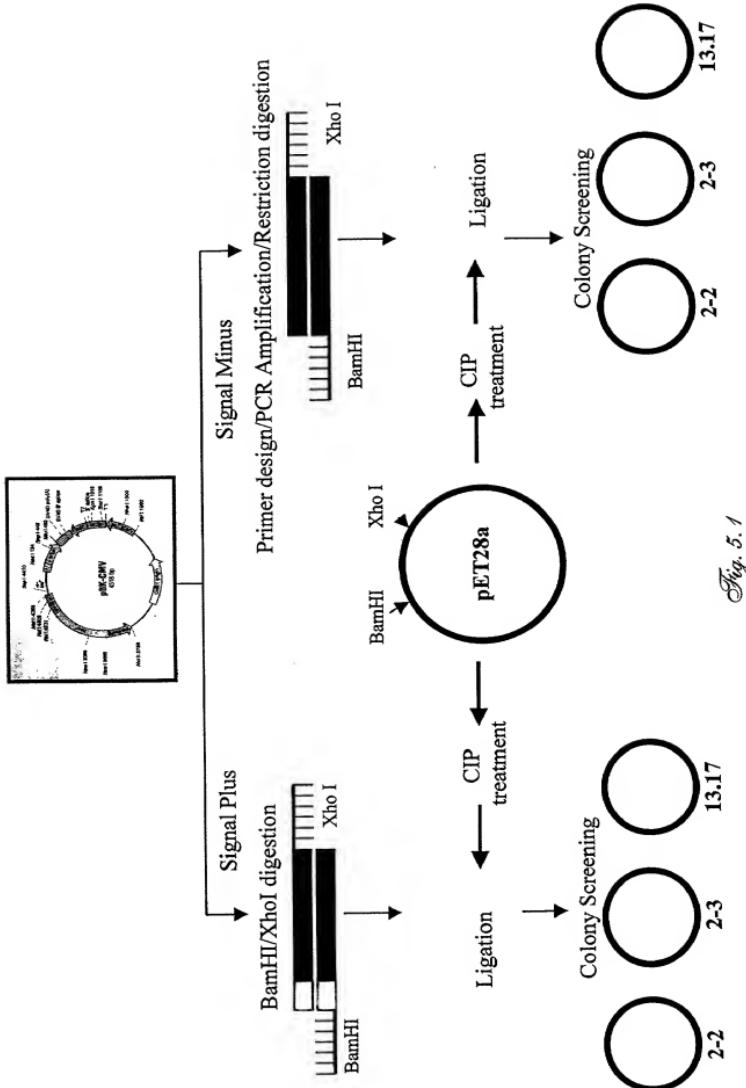


Fig. 5. 1

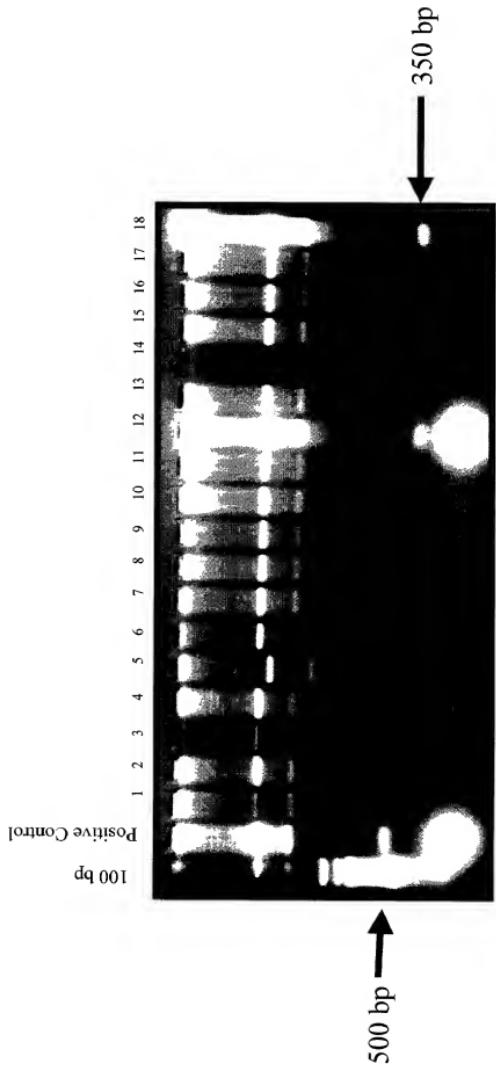


Fig. 5.2

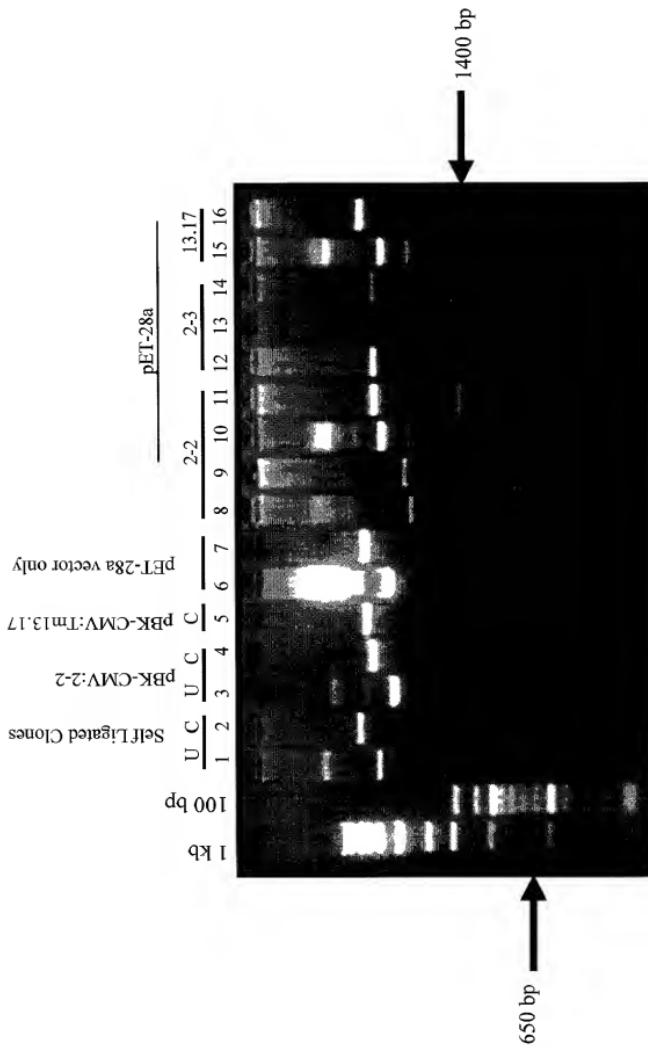


Fig. 5.3

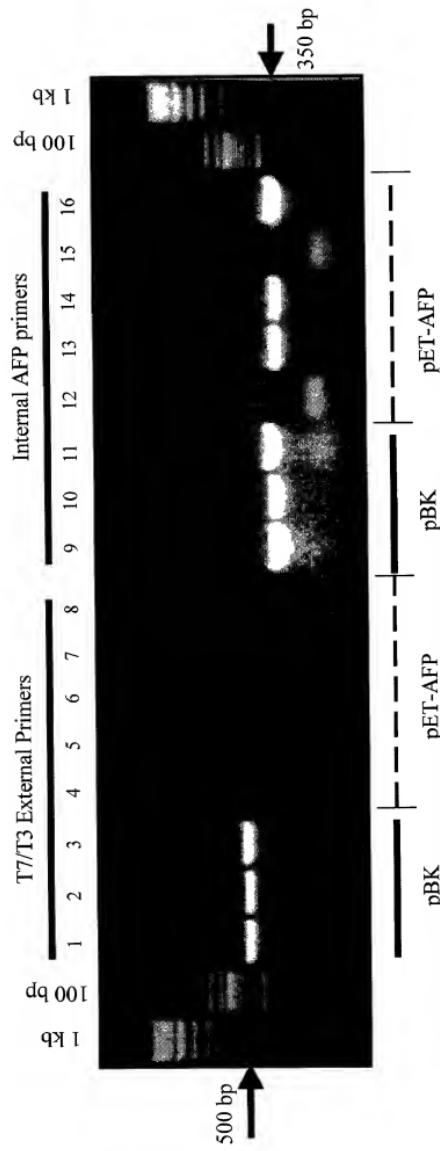
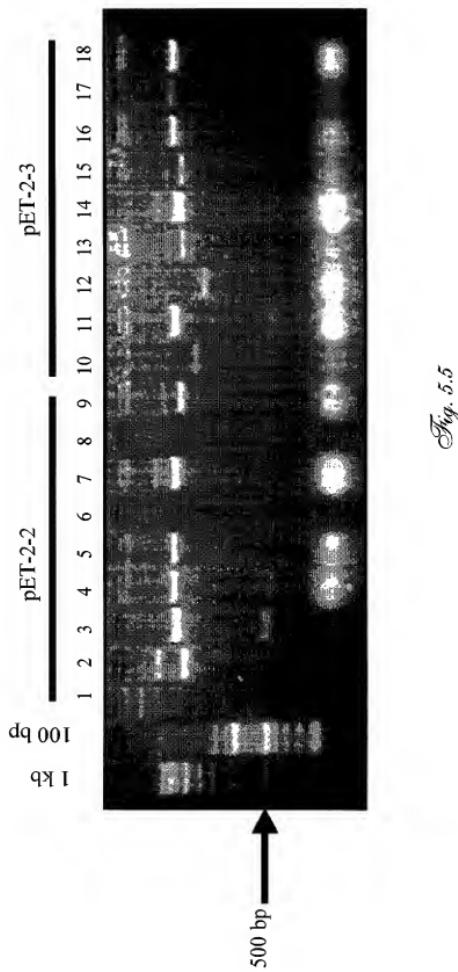


Fig. 5.4



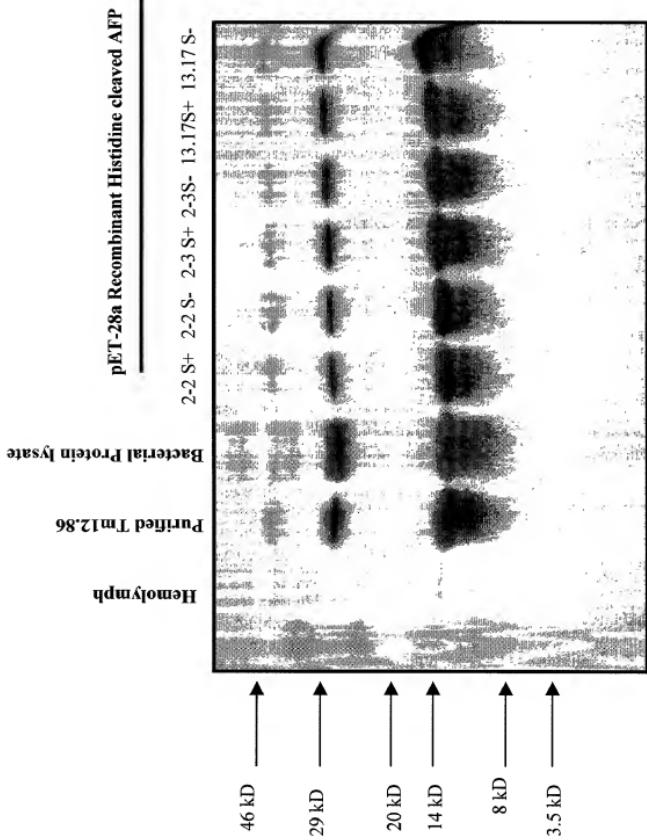


Fig. 5.6

D 962920 500

His-tagged Clone 2 with signal sequence

TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTGT TTACITTAAG	50
His-tag Start Codon	
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC	96
Met Gly Ser Ser His His His His His His Ser	
-55	
AGC GGC CTG GTG CGG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT	141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly	
-45 -40 -35	
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG	186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met	
-30 -25 -20	
AAA CTC CTC TTG TGT TTC GGC GCC ATC GTC ATC GGA GCT	231
Lys Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala	
-15 -10 -5	
N-terminal of mature AFP	
CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG AAG ATC AGC	276
Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser	
1 5 10	
AAA GAA TGC CAG GTG TCC GGA GTG TCC CAA GAG AGC ATC GAC	321
Lys Glu Cys Gln Gln Val Ser Gly Val Ser Glu Glu Thr Ile Asp	
15 20 25	
AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG	366
Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys	
30 35 40	
CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC	411
His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala	
45 50 55	
GGA GAC ACC AAT GTG GAG GCA CTC AAA GCC AAG CTG AAG CAT GTG	456
Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val	
60 65 70	
GCC AGC GAC GAA GAG GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC	501
Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val	
75 80 85	
AGG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT	546
Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys	
90 95 100	
Stop Codon	
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TGTGTTGTA	595
Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp *	
105 110 115	
Polyadenylation signal Poly-A tail	
TTTGACTGAA TTTCGACAAT <u>AAAGGTATA</u> TCGTATGTAA AAAAAAAA	645
AAAAAACTCG AGCACCCACCA CCACCAACAC TGAGAT	681

Fig. 5.7

His-tagged clone 2.2 without signal sequence

TGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTGT TTACTTTAG	50
His-tag Start Codon	
AAGGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC	96
Met Gly Ser Ser His His His His His Ser	
-30 -25	
AGC GGC CTG GTG CGG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT	141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly	
-20 -15 -10	
N-terminal of mature AFP	
GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG	186
Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln	
-5 1 5	
AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG	231
Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val	
10 15 20	
TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT	276
Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp	
25 30 35	
GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT	321
Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr	
40 45 50	
GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA	366
Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys	
55 60 65	
GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAG GTG GAC AAG ATC	411
Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile	
70 75 80	
GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT	456
Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala	
85 90 95	
TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT	501
Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser	
100 105 110	
Stop Codon	
CCT ATT GAT TAA CTCGAGCACCCACCA CCACTGAGAT	543
Pro Ile Asp *	
115	

Fig. 5.8

His-tagged clone 2 3 with signal sequence

TTGGTAGGGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG	50
His-tag Start Codon	
AAGGAGAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC	96
Met Gly Ser Ser His His His His His His His Ser	
-55 -50	
AGC GGC CTG GTG CGG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT	141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly	
-45 -40 -35	
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG	186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met	
-30 -25 -20	
AAA CTC CTC TTG TGC TTT GCT TTC GGC GCC ATC GTC ATC GGA GCT	231
Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala	
-15 -10 -5	
N-terminal of Mature AFP	
CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC	276
Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser	
1 5 10	
AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC	321
Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp	
15 20 25	
AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG	366
Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys	
30 35 40	
CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GGC	411
His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala	
45 50 55	
GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG	456
Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val	
60 65 70	
GCC AGC GAC GAA GAA GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC	501
Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val	
75 80 85	
AAG AAG GCC ACA CCA GAG GAA ACC GCT TAT GAC ACC TTC AAG TGT	546
Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys	
90 95 100	
Stop Codon	
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTGTAA	595
Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp *	
105 110 115	
Polyadenylation signal Poly-A tail	
TTTGACTGAA TTGACTGAA AAAGGTACTA TGTTATGAA AAAA.....	645
AAAAAAACTC GAGGACCAAC ACCACACCA CTGAGAT	682

Fig. 5.9

His-tagged Clone 2.3 without signal sequence

TTGTTAGCGG ATGGAATCC CTCGTAGGGG ATAATTTGT TTACTTTAAG	50
His-tag Start Codon	
AAGGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC	96
Met Gly Ser Ser His His His His His Ser	
-30 -25	
AGC GGC CTG GTG CGC CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT	141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly	
-20 -15 -10	
N-terminal of mature AFP	
GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG	186
Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln	
-5 1 5	
AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG	231
Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val	
10 15 20	
TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT	276
Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp	
25 30 35	
GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT	321
Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr	
40 45 50	
GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA	366
Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys	
55 60 65	
GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAA GTG GAC AAG ATC	411
Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile	
70 75 80	
GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT	456
Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala	
85 90 95	
TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT	501
Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser	
100 105 110	
Stop Codon	
CCT ATT GAT TAA CTCGAGCACC ACCACCA CCACTGAGAT	543
Pro Ile Asp *	
115	

Fig. 5.10

His-tagged Tm 13 17 with signal sequence

		50
	His-tag Start Codon	
AAGGAGATAT	ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC	96
	Met Gly Ser Ser His His His His His Ser	
-65	-60	-55
AGC GCC CTC GTG CGG GCC GGC AGC CAT ATG GCT AGC ATG ATG GGT		141
Ser Gly Leu Val	Pro Arg Gly Ser His Met Ala Ser Met Thr Gly	
-50	-45	-40
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC TGG ATC CAA AGA ATT		186
Gly Gln Met Gly Arg Gly Ser Glu Phe Trp Ile Gln Arg Ile		
-35	-30	-25
	AFP Start Codon	
CGG CAC GAG ACT ACT AAG ATG AAG TTG CTC TGT TGT CTA ATC TCC		231
Arg His Glu Thr Thr Lys Met Leu Cys Cys Leu Ile Ser		
-20	-15	-10
	N-terminal of mature AFP	
CTC ATT CTG TTG GTC ACA GGT CAC GCC CTG ACC GAG GCA CAA ATT		276
Leu Ile Leu Leu Val Thr Val Gln Ala Leu Thr Glu Ala Gln Ile		
-5	1	5
GAG AAA CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA ATG GGA		321
Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys Cys Asn Glu Ser Gly		
10	15	20
GTG TCG CAA GAG ATC ATA ACC AAA GGT CGC AAC AGC GGT GAC TGG GAG		366
Val Ser Gin Ile Ile Thr Lys Ala Arg Asn Glu Trp Glu		
25	30	35
GAC GAT CCT AAA CTG AAA CGC CAA GGT TTT TGC GTG GGC AGG AAC		411
Asp Asp Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn		
40	45	50
GCC GGT CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG		456
Ala Gly Leu Ala Thr Glu Ser Gly Val Val Val Asp Val Leu		
55	60	65
AGG GAG AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG		501
Arg Glu Lys Val Arg Lys Val Thr Asn Asp Glu Glu Thr Glu		
70	75	80
AAA ATC ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG		546
Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu		
85	90	95
ACG GTG TTC ATT ACT TTC AAA TGT GTG ATC ATG AAA AAC GAA CCA AGG		595
Arg Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys		
100	105	110
	Stop Codon	
TTC TCA CCA GTT GAT TGA ACCACCAAGA CTAGTAGATG GTTCAAATGG		643
Pho Ser Pro Val Asp *		
115		
	Polyadenylation signal Poly-A tail	
TGTGCTTAC ATATTTAAAT AAATGTTTC TGTATGAAAA AAAAAAAA		693
AAAAAAAAAA AACTCTGAGAG TATTCTAGAG CGCCCGCGGG CCCATCGTTT		743
TCCACCCCTC GAGCACCC ACCACCCCA CTGAGAT		777

His-tagged Tm 13.17 without signal sequence

TGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTGT TTACTTTAAG	50
His-tag Start Codon	
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC	96
Met Gly Ser Ser His His His His His His Ser	
-30 -25	
AGC GGC CTG GTG CGG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT	141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly	
-20 -15 -10	
N-terminal of mature AFP	
GGA CAG CAA ATG GGT CGC GGC CTG ACC GAG GCA CAA ATT GAG AAA	186
Gly Gln Gln Met Gly Arg Gly Leu Thr Glu Ala Gln Ile Glu Lys	
-5 1 5	
CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA GTG TCG	231
Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly Val Ser	
10 15 20	
CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG GAC GAT	276
Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu Asp Asp	
25 30 35	
CTT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC GCC GGT	321
Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn Ala Gly	
40 45 50	
CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG AGG GAG	366
Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu Arg Glu	
55 60 65	
AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG AAA ATC	411
Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu Lys Ile	
70 75 80	
ATC AAT AAG TGC GTC AAG AGA GAT ACT GTT GAA GAG ACG GTG	456
Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu Thr Val	
85 90 95	
TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG TTC TCA	501
Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys Phe Ser	
100 105 110	
Stop Codon	
CCA GTT GAT TGA CTCGAGCACC ACCACCCACCA CCACTGAGAT	543
Pro Val Asp *	
115	

Fig. 5.12

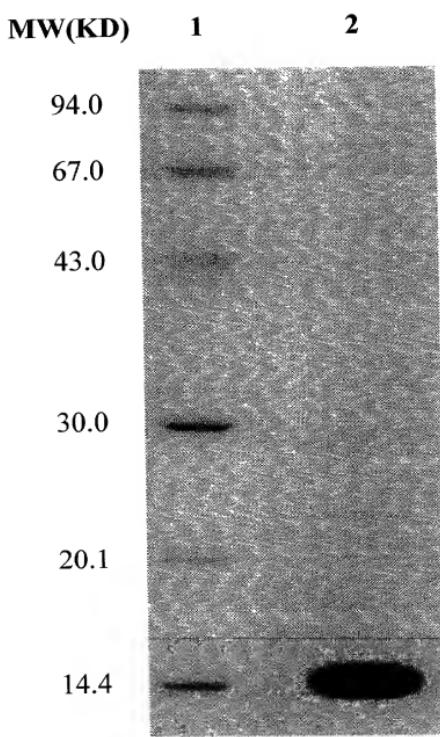


Fig. 6.0

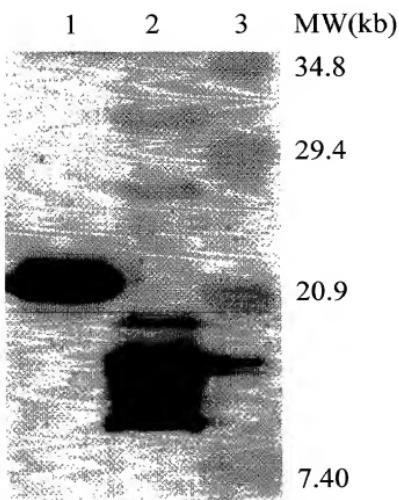


Fig. 6.1

09876796, 02125

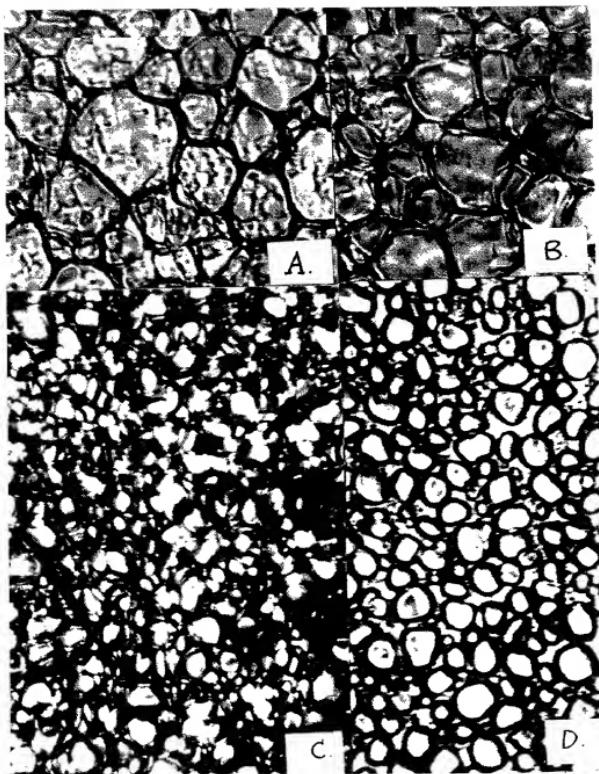


Fig. 6.2

Tm 13.17 S-graph data

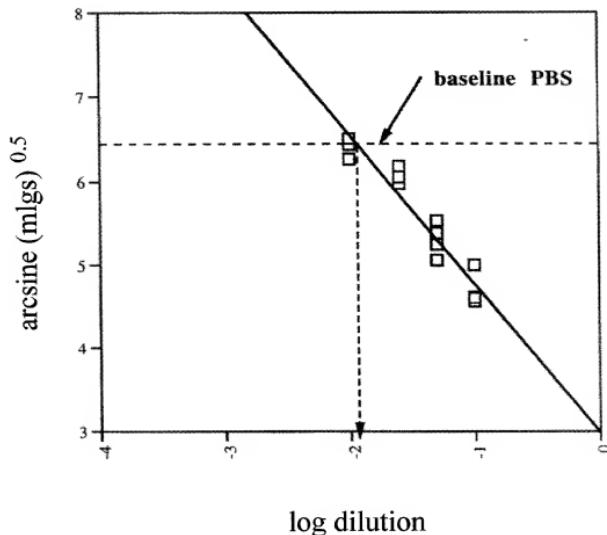


Fig. 6.3

ONE LETTER	NAME	THREE LETTER	CHEMICAL CLASS	HYDROPHOBICITY	Reactivity
A	Alanine	Ala	aliphatic	mod. hydrophobic	low
B	Asp or Asn	Asx			
C	Cysteine	Cys	sulphydryl	hydrophobic	high
D	Aspartic Acid	Asp	acidic	highly hydrophilic	high
E	Glutamic Acid	Glu	acidic	highly hydrophilic	high
F	Phenylalanine	Phe	aromatic	highly hydrophobic	low
G	Glycine	Gly	aliphatic	mod. hydrophobic	low
H	Histidine	His	basic, imidazole	highly hydrophilic	high
I	Isoleucine	Ile	aliphatic	hydrophobic	low
J					
K	Lysine	Lys	basic	highly hydrophilic	high
L	Leucine	Leu	aliphatic	hydrophobic	low
M	Methionine	Met	sulphydryl	hydrophobic	low
N	Asparagine	Asn	amide, acidic derived	hydrophilic	high
O					
P	Proline	Pro	aliphatic, cyclic, imino	mod. hydrophilic	low
Q	Glutamine	Gln	amide, acidic derived	hydrophilic	high
R	Arginine	Arg	basic	highly hydrophilic	high
S	Serine	Ser	aliphatic hydroxyl	hydrophilic	high
T	Threonine	Thr	aliphatic hydroxyl	hydrophilic	high
U					
V	Valine	Val	aliphatic	hydrophobic	low
W	Tryptophan	Trp	aromatic	highly hydrophobic	low
X					
Y	Tyrosine	Tyr	aromatic	mod. hydrophilic	high
Z	Glu or Gln	Glx			
		ACD	Any Acidic		
		ALP	Any Aliphatic		
		ALH	Any Aliphatic Hydroxyl		
		ARO	Any Aromatic		
		BAS	Any Basic		
		HY-	Hydrophobic		
		HY+	Hydrophilic		

Fig. 7.1

Fig. 7.2

Position	Tm12 84-2-2	Tm12 84-2-3	Tm12 84-2-4	Tm12 84-2-5	Consensus of	Tm13 17	Consensus with	AFP-3	Consensus w/o
116	C	A	C	C	C	A	N	G	N
119	A	G	A	G	A	A	N	G	N
120	G	T	G	T	G	G	N	A	O
121	T	T	T	T	T	T	R	T	R
122	C	C	C	C	C	C	R	R	R
124	T	G	G	G	G	G	D	G	G
125	C	C	C	C	C	C	V	G	V
126	T	T	T	T	T	T	G	G	G
127	C	G	G	G	G	G	G	G	G
128	G	G	G	G	G	G	G	G	G
129	A	T	T	T	T	T	G	G	G
130	G	T	T	T	T	T	G	G	G
131	T	G	G	G	G	G	G	G	G
132	C	C	C	C	C	C	G	G	G
134	A	A	A	A	A	A	G	G	G
135	A	A	A	A	A	A	G	G	G
136	T	C	C	C	C	C	G	G	G
137	C	C	C	C	C	C	G	G	G
138	C	C	C	C	C	C	G	G	G
139	A	A	A	A	A	A	G	G	G
140	G	A	A	A	A	A	G	G	G
141	A	C	C	C	C	C	G	G	G
143	G	A	A	A	A	A	G	G	G
145	A	T	T	T	T	T	G	G	G
146	T	C	C	C	C	C	G	G	G
147	C	G	G	G	G	G	G	G	G
148	G	A	A	A	A	A	G	G	G
149	A	A	A	A	A	A	G	G	G
150	T	T	T	T	T	T	G	G	G
151	C	A	A	A	A	A	G	G	G
152	A	A	A	A	A	A	G	G	G
153	A	G	G	G	G	G	G	G	G
154	T	C	C	C	C	C	G	G	G
155	C	G	G	G	G	G	G	G	G
156	G	A	A	A	A	A	G	G	G
157	A	A	A	A	A	A	G	G	G
158	T	T	T	T	T	T	G	G	G
159	C	C	C	C	C	C	G	G	G
160	G	G	G	G	G	G	G	G	G
161	A	A	A	A	A	A	G	G	G
162	T	T	T	T	T	T	G	G	G
163	C	C	C	C	C	C	G	G	G
164	G	G	G	G	G	G	G	G	G
165	A	A	A	A	A	A	G	G	G
166	T	T	T	T	T	T	G	G	G
167	C	C	C	C	C	C	G	G	G
168	G	G	G	G	G	G	G	G	G
169	A	A	A	A	A	A	G	G	G
170	T	T	T	T	T	T	G	G	G
171	C	C	C	C	C	C	G	G	G
172	G	G	G	G	G	G	G	G	G
173	A	A	A	A	A	A	G	G	G
174	T	T	T	T	T	T	G	G	G
175	C	C	C	C	C	C	G	G	G
176	G	G	G	G	G	G	G	G	G
177	A	A	A	A	A	A	G	G	G
178	T	T	T	T	T	T	G	G	G
179	C	C	C	C	C	C	G	G	G
180	G	G	G	G	G	G	G	G	G
181	A	A	A	A	A	A	G	G	G
182	T	T	T	T	T	T	G	G	G
183	C	C	C	C	C	C	G	G	G
184	G	G	G	G	G	G	G	G	G
185	A	A	A	A	A	A	G	G	G
186	T	T	T	T	T	T	G	G	G
187	C	C	C	C	C	C	G	G	G
188	G	G	G	G	G	G	G	G	G
189	A	A	A	A	A	A	G	G	G
190	T	T	T	T	T	T	G	G	G
191	C	C	C	C	C	C	G	G	G
192	G	G	G	G	G	G	G	G	G
193	A	A	A	A	A	A	G	G	G
194	T	T	T	T	T	T	G	G	G
195	C	C	C	C	C	C	G	G	G
196	G	G	G	G	G	G	G	G	G
197	A	A	A	A	A	A	G	G	G
198	T	T	T	T	T	T	G	G	G
199	C	C	C	C	C	C	G	G	G
200	G	G	G	G	G	G	G	G	G
201	A	A	A	A	A	A	G	G	G
202	T	T	T	T	T	T	G	G	G
203	C	C	C	C	C	C	G	G	G
204	G	G	G	G	G	G	G	G	G
205	A	A	A	A	A	A	G	G	G
206	T	T	T	T	T	T	G	G	G
207	C	C	C	C	C	C	G	G	G
208	G	G	G	G	G	G	G	G	G
209	A	A	A	A	A	A	G	G	G
210	T	T	T	T	T	T	G	G	G
211	C	C	C	C	C	C	G	G	G
212	G	G	G	G	G	G	G	G	G
213	A	A	A	A	A	A	G	G	G
214	T	T	T	T	T	T	G	G	G
215	C	C	C	C	C	C	G	G	G
216	G	G	G	G	G	G	G	G	G
217	A	A	A	A	A	A	G	G	G
218	T	T	T	T	T	T	G	G	G
219	C	C	C	C	C	C	G	G	G
220	G	G	G	G	G	G	G	G	G
221	A	A	A	A	A	A	G	G	G
222	T	T	T	T	T	T	G	G	G
223	C	C	C	C	C	C	G	G	G
224	G	G	G	G	G	G	G	G	G
225	A	A	A	A	A	A	G	G	G
226	T	T	T	T	T	T	G	G	G
227	C	C	C	C	C	C	G	G	G
228	G	G	G	G	G	G	G	G	G
229	A	A	A	A	A	A	G	G	G
230	T	T	T	T	T	T	G	G	G
231	C	C	C	C	C	C	G	G	G
232	G	G	G	G	G	G	G	G	G
233	A	A	A	A	A	A	G	G	G
234	T	T	T	T	T	T	G	G	G
235	C	C	C	C	C	C	G	G	G
236	G	G	G	G	G	G	G	G	G
237	A	A	A	A	A	A	G	G	G
238	T	T	T	T	T	T	G	G	G
239	C	C	C	C	C	C	G	G	G
240	G	G	G	G	G	G	G	G	G

Fig. 7.2 cont.

Position	Tr12 84-2-2	Tr12 84-2-3	Tr12 84-3-4	Tr12 84-3-9	Tr12 84-7-5	Concensus of	Tr13 17	Concensus with	APP-3	Concensus with
236	A	A	A	A	A	A	A	A	N	N
237	G	G	G	G	G	G	G	G	G	G
238	C	C	C	C	C	C	C	C	N	N
239	G	G	G	G	G	G	G	G	N	N
240	G	G	G	G	G	G	G	G	N	N
241	G	G	G	G	G	G	G	G	N	N
242	G	G	G	G	G	G	G	G	N	N
243	G	G	G	G	G	G	G	G	N	N
244	G	G	G	G	G	G	G	G	N	N
245	G	G	G	G	G	G	G	G	N	N
246	G	G	G	G	G	G	G	G	N	N
247	G	G	G	G	G	G	G	G	N	N
248	C	C	C	C	C	C	C	C	N	N
249	A	A	A	A	A	A	A	A	N	N
250	A	A	A	A	A	A	A	A	N	N
251	T	T	T	T	T	T	T	T	N	N
252	G	G	G	G	G	G	G	G	N	N
253	T	T	T	T	T	T	T	T	N	N
254	G	G	G	G	G	G	G	G	N	N
255	G	G	G	G	G	G	G	G	N	N
256	G	G	G	G	G	G	G	G	N	N
257	G	G	G	G	G	G	G	G	N	N
258	G	G	G	G	G	G	G	G	N	N
259	G	G	G	G	G	G	G	G	N	N
260	T	T	T	T	T	T	T	T	N	N
261	C	C	C	C	C	C	C	C	N	N
262	T	T	T	T	T	T	T	T	N	N
263	C	C	C	C	C	C	C	C	N	N
264	A	A	A	A	A	A	A	A	N	N
265	A	A	A	A	A	A	A	A	N	N
266	A	A	A	A	A	A	A	A	N	N
267	G	G	G	G	G	G	G	G	N	N
268	G	G	G	G	G	G	G	G	N	N
269	C	C	C	C	C	C	C	C	N	N
270	A	A	A	A	A	A	A	A	N	N
271	A	A	A	A	A	A	A	A	N	N
272	G	G	G	G	G	G	G	G	N	N
273	T	T	T	T	T	T	T	T	N	N
274	G	G	G	G	G	G	G	G	N	N
275	T	T	T	T	T	T	T	T	N	N
276	G	G	G	G	G	G	G	G	N	N
277	A	A	A	A	A	A	A	A	N	N
278	G	G	G	G	G	G	G	G	N	N
279	G	G	G	G	G	G	G	G	N	N
280	C	C	C	C	C	C	C	C	N	N
281	A	A	A	A	A	A	A	A	N	N
282	A	A	A	A	A	A	A	A	N	N
283	G	G	G	G	G	G	G	G	N	N
284	G	G	G	G	G	G	G	G	N	N
285	T	T	T	T	T	T	T	T	N	N
286	G	G	G	G	G	G	G	G	N	N
287	T	T	T	T	T	T	T	T	N	N
288	G	G	G	G	G	G	G	G	N	N
289	A	A	A	A	A	A	A	A	N	N
290	G	G	G	G	G	G	G	G	N	N
291	G	G	G	G	G	G	G	G	N	N
292	G	G	G	G	G	G	G	G	N	N
293	G	G	G	G	G	G	G	G	N	N
294	G	G	G	G	G	G	G	G	N	N
295	G	G	G	G	G	G	G	G	N	N
296	G	G	G	G	G	G	G	G	N	N
297	G	G	G	G	G	G	G	G	N	N
298	G	G	G	G	G	G	G	G	N	N
299	G	G	G	G	G	G	G	G	N	N
300	A	A	A	A	A	A	A	A	N	N
301	A	A	A	A	A	A	A	A	N	N
302	T	T	T	T	T	T	T	T	N	N
303	G	G	G	G	G	G	G	G	N	N
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305	T	T	T	T	T	T	T	T	N	N
306	G	G	G	G	G	G	G	G	N	N
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312	T	T	T	T	T	T	T	T	N	N
313	G	G	G	G	G	G	G	G	N	N
314	T	T	T	T	T	T	T	T	N	N
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316	T	T	T	T	T	T	T	T	N	N
317	G	G	G	G	G	G	G	G	N	N
318	G	G	G	G	G	G	G	G	N	N
319	G	G	G	G	G	G	G	G	N	N
320	G	G	G	G	G	G	G	G	N	N
321	G	G	G	G	G	G	G	G	N	N
322	G	G	G	G	G	G	G	G	N	N
323	G	G	G	G	G	G	G	G	N	N
324	A	A	A	A	A	A	A	A	N	N
325	A	A	A	A	A	A	A	A	N	N
326	G	G	G	G	G	G	G	G	N	N
327	G	G	G	G	G	G	G	G	N	N
328	T	T	T	T	T	T	T	T	N	N
329	G	G	G	G	G	G	G	G	N	N
330	T	T	T	T	T	T	T	T	N	N
331	G	G	G	G	G	G	G	G	N	N
332	T	T	T	T	T	T	T	T	N	N
333	C	C	C	C	C	C	C	C	N	N
334	T	T	T	T	T	T	T	T	N	N
335	A	A	A	A	A	A	A	A	N	N
336	G	G	G	G	G	G	G	G	N	N
337	G	G	G	G	G	G	G	G	N	N
338	A	A	A	A	A	A	A	A	N	N
339	A	A	A	A	A	A	A	A	N	N
340	G	G	G	G	G	G	G	G	N	N
341	G	G	G	G	G	G	G	G	N	N
342	A	A	A	A	A	A	A	A	N	N
343	A	A	A	A	A	A	A	A	N	N
344	C	C	C	C	C	C	C	C	N	N
345	A	A	A	A	A	A	A	A	N	N
346	G	G	G	G	G	G	G	G	N	N
347	G	G	G	G	G	G	G	G	N	N
348	A	A	A	A	A	A	A	A	N	N
349	G	G	G	G	G	G	G	G	N	N
350	G	G	G	G	G	G	G	G	N	N
351	G	G	G	G	G	G	G	G	N	N
352	G	G	G	G	G	G	G	G	N	N

Fig. 7.2 cont.

Fig. 7.2 cont.

Position	Tm12 84-2-2	Tm12 84-2-3	Tm12 84-3-4	Tm12 84-3-9	Tm12 84-7-5	Consensus of	Tm13 17	Consensus with	S1	Consensus with	AFP-3	Consensus with
472	A	A	A	A	A	A	A	A	A	A	A	A
473	A	T	A	T	A	A	A	A	A	A	T	A
474	T	T	A	T	A	A	A	A	A	A	A	A
475	A	A	A	A	A	A	A	A	A	A	A	A
476	A	A	A	A	A	A	A	A	A	A	A	A
477	A	A	A	A	A	A	A	A	A	A	A	A
478	G	G	G	G	G	G	G	G	G	G	N	N
479	G	T	T	T	T	T	T	T	T	T	T	N
480	A	A	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	ACT	N
481	A	T	T	T	T	T	T	T	T	T	G	ACT
482	T	T	A	T	A	T	T	T	T	T	A	AT
483	C	C	G	G	G	G	G	G	G	G	A	R
484	G	G	G	G	G	G	G	G	G	G	G	GA
485	T	T	T	T	T	T	T	T	T	T	T	AT
486	C	C	C	C	C	C	C	C	C	C	C	TA
487	G	G	G	G	G	G	G	G	G	G	G	TA
488	T	T	T	T	T	T	T	T	T	T	T	TA
489	T	T	T	T	T	T	T	T	T	T	T	TA
490	A	A	A	A	A	A	A	A	A	A	A	TA
491	T	T	T	T	T	T	T	T	T	T	T	TA
492	G	G	G	G	G	G	G	G	G	G	G	TA
493	A	A	A	A	A	A	A	A	A	A	A	TA
494	A	A	A	A	A	A	A	A	A	A	A	TA
495	A	A	A	A	A	A	A	A	A	A	A	TA
496	A	A	A	A	A	A	A	A	A	A	A	TA
497	A	A	A	A	A	A	A	A	A	A	A	TA
498	A	A	A	A	A	A	A	A	A	A	A	TA
499	A	A	A	A	A	A	A	A	A	A	A	TA
500	A	A	A	A	A	A	A	A	A	A	A	TA
501	A	A	A	A	A	A	A	A	A	A	A	TA
502	A	A	A	A	A	A	A	A	A	A	A	TA
503	A	A	A	A	A	A	A	A	A	A	A	TA
504	A	A	A	A	A	A	A	A	A	A	A	TA
505	A	A	A	A	A	A	A	A	A	A	A	TA
506	A	A	A	A	A	A	A	A	A	A	A	TA
507	A	A	A	A	A	A	A	A	A	A	A	TA
508	A	A	A	A	A	A	A	A	A	A	A	TA
509	A	A	A	A	A	A	A	A	A	A	A	TA
510	A	A	A	A	A	A	A	A	A	A	A	TA
511	A	A	A	A	A	A	A	A	A	A	A	TA
512												

Fig. 7.2 cont.

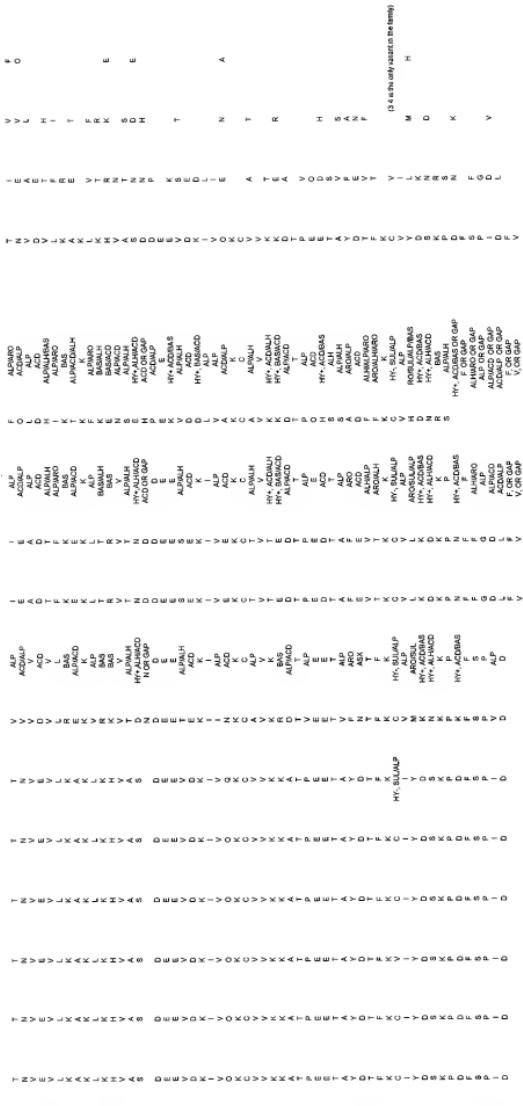


Fig. 7.3 cont.

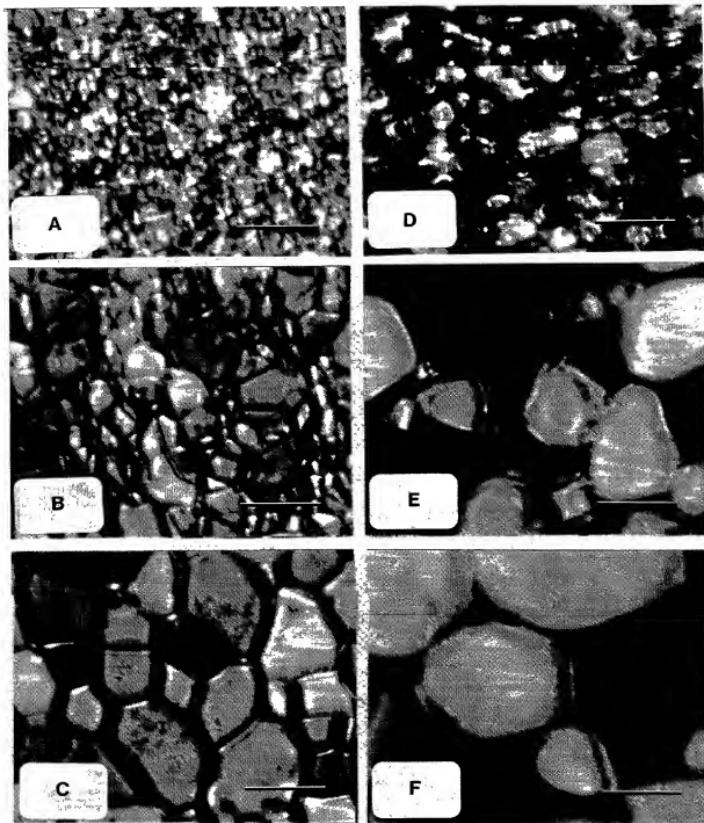


Fig. 8.0

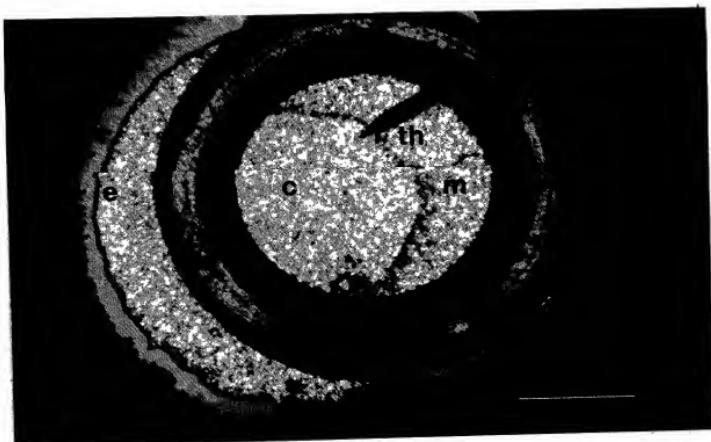


Fig. 8.1a

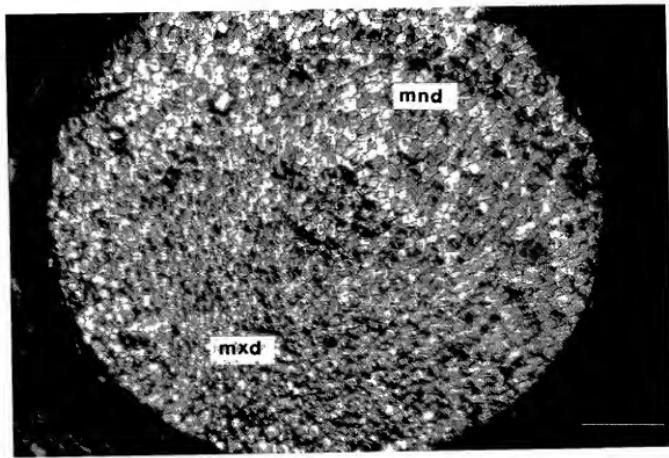
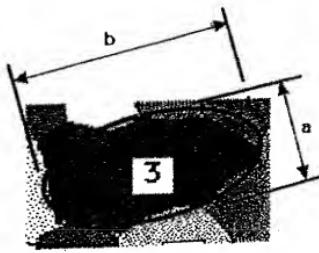
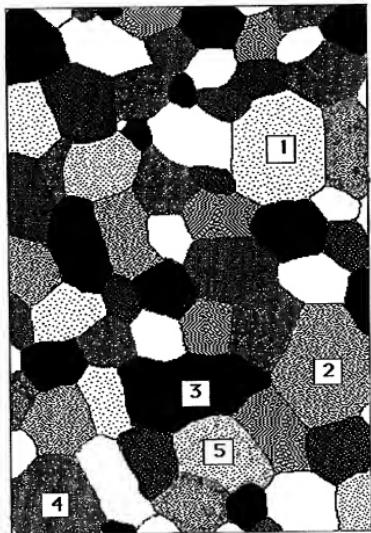


Fig. 8.1b



grain area = 0.25ab

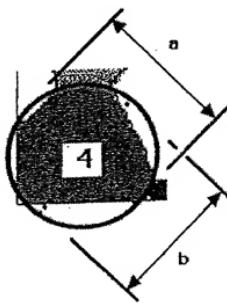


Fig. 8.2

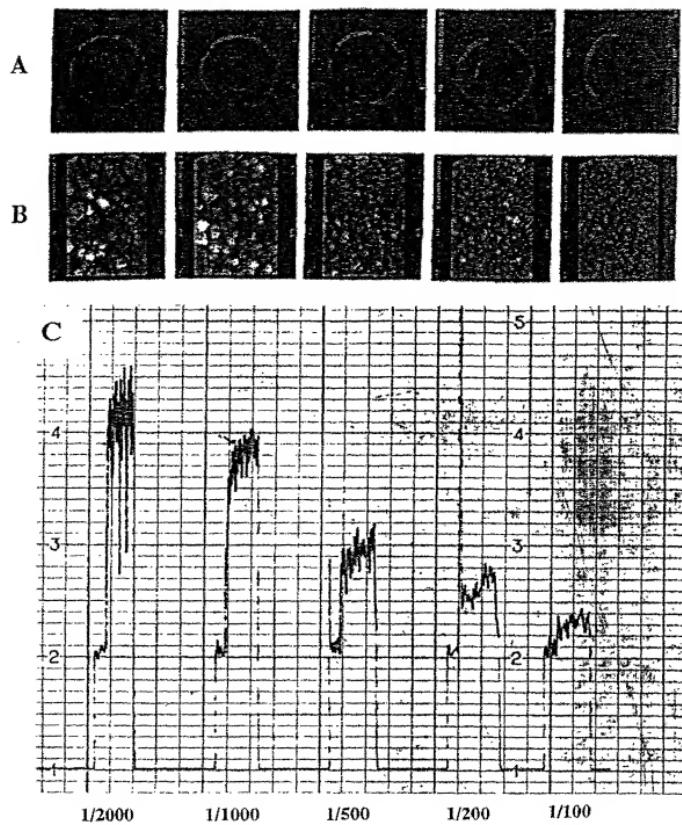


Fig. 8.3

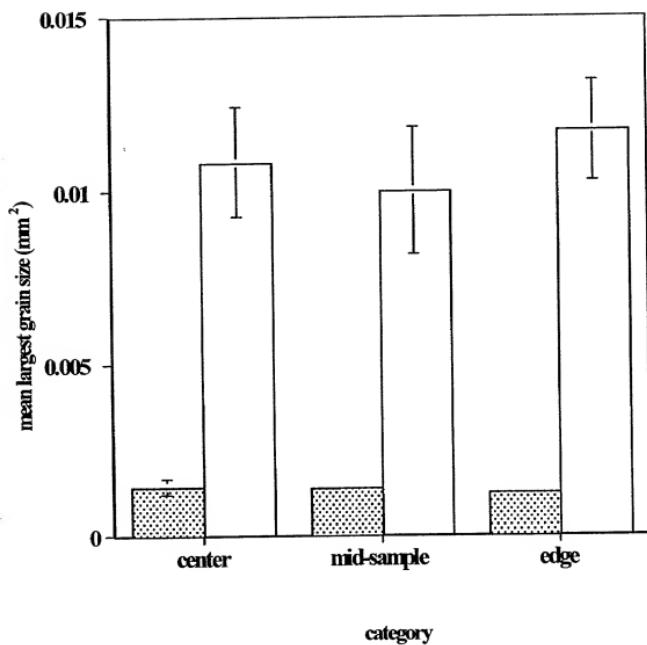


Fig. 8.4a

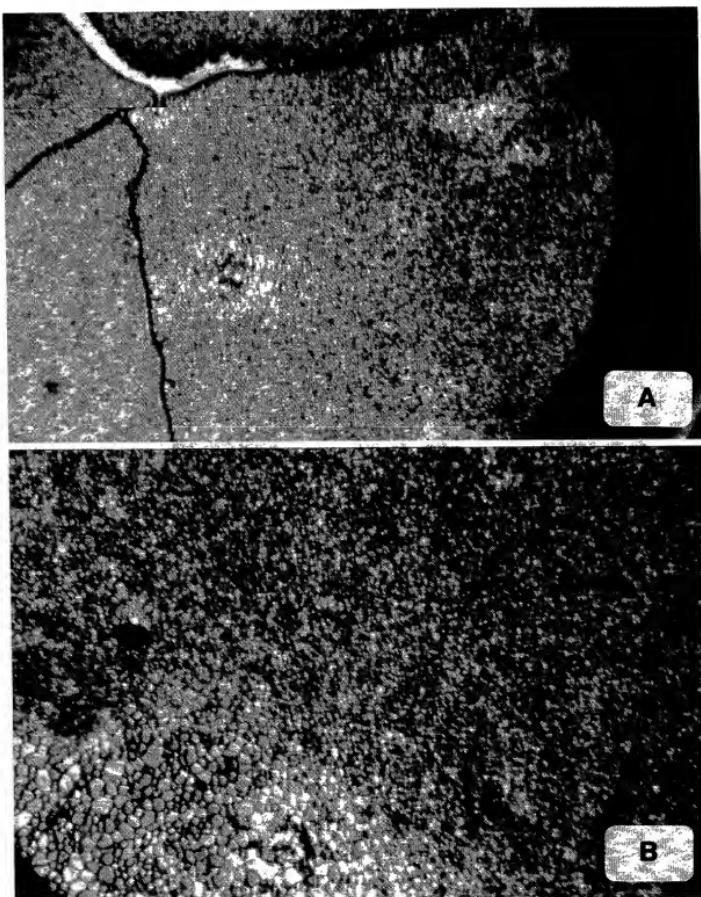


Fig. 8.4b

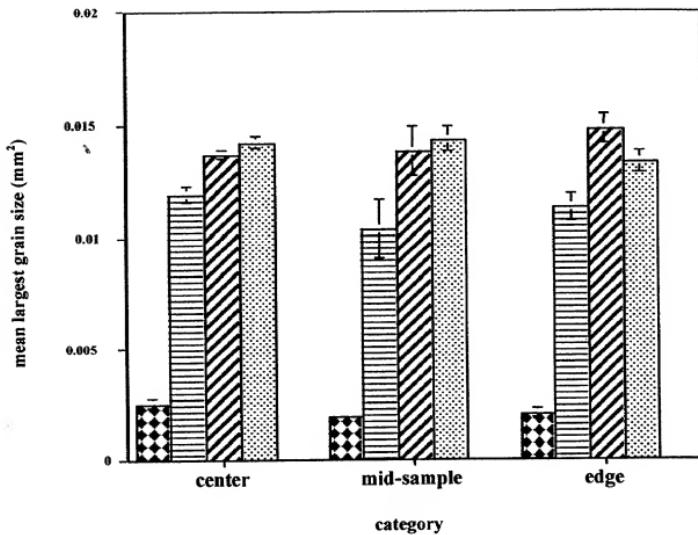


Fig. 8.5a

2020-02-27 09:28:56

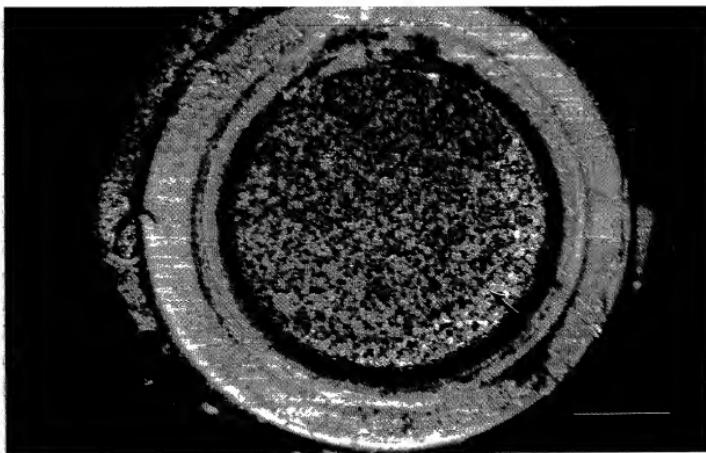


Fig. 8.5b

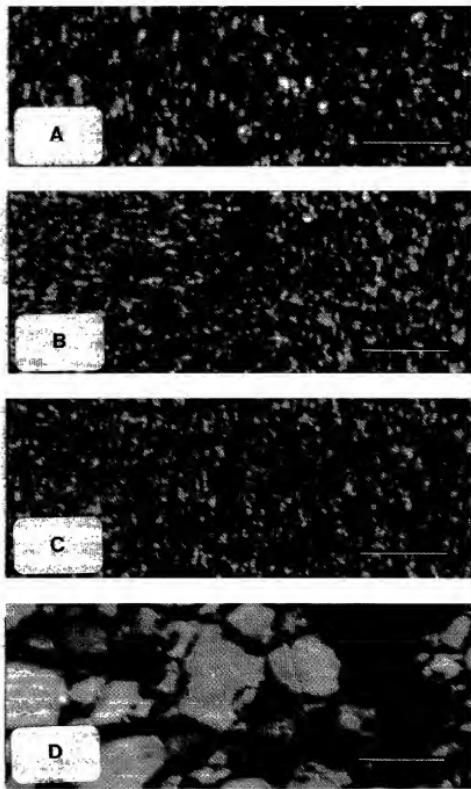


Fig. 8.6

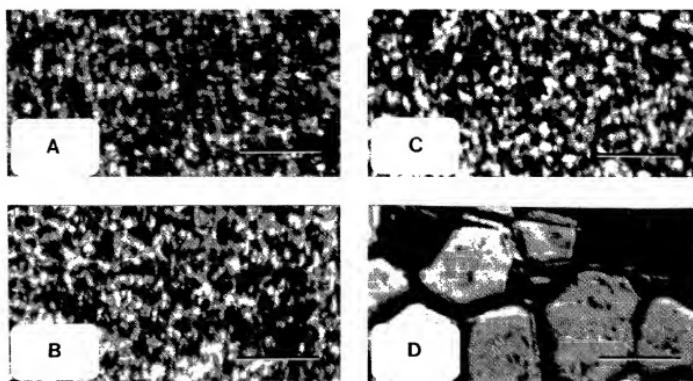


Fig. 8.7

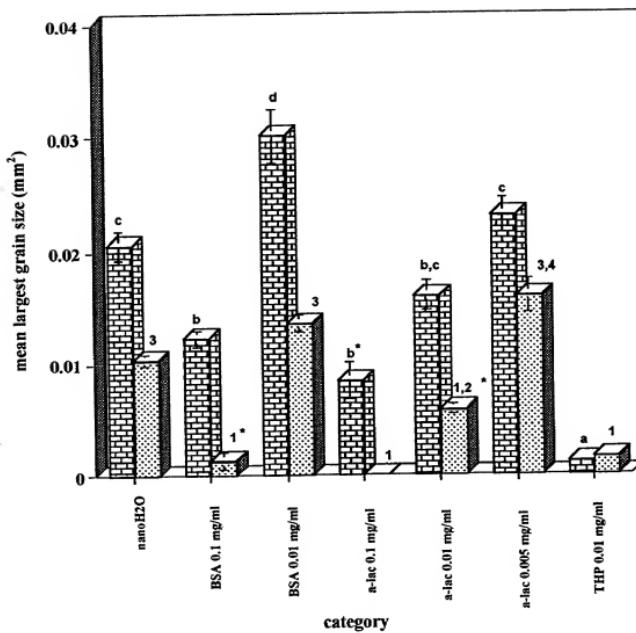


Fig. 8.8

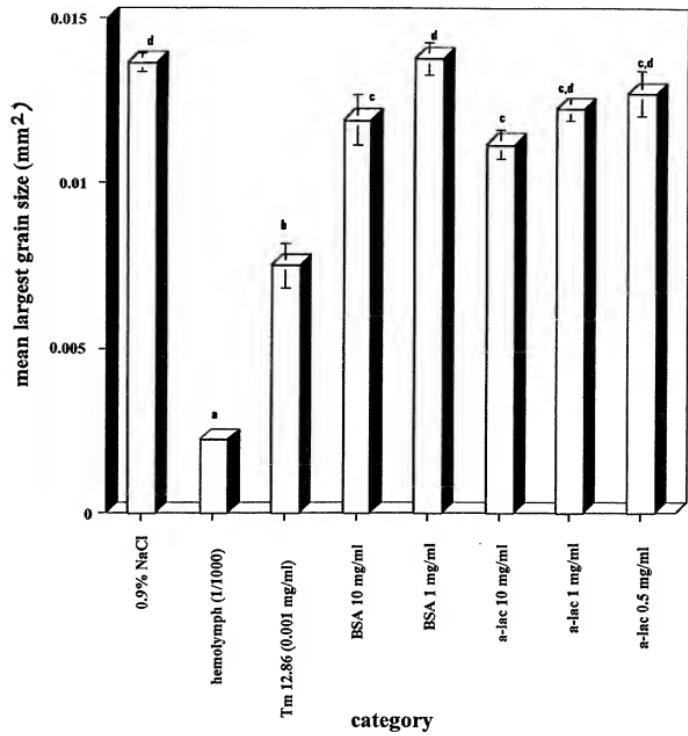


Fig. 8.9

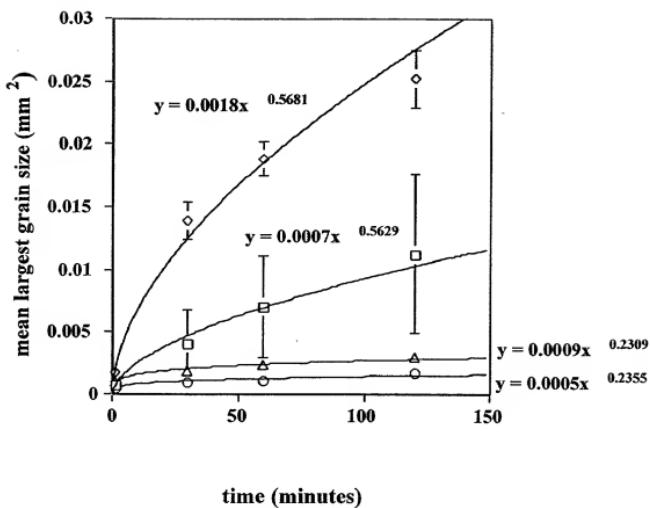


Fig. 8.10

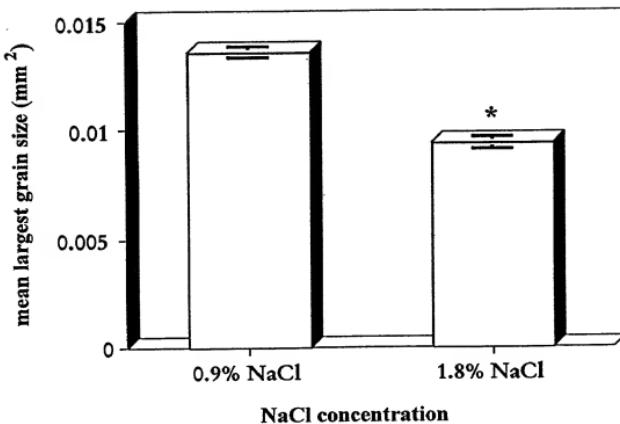


Fig. 8.11

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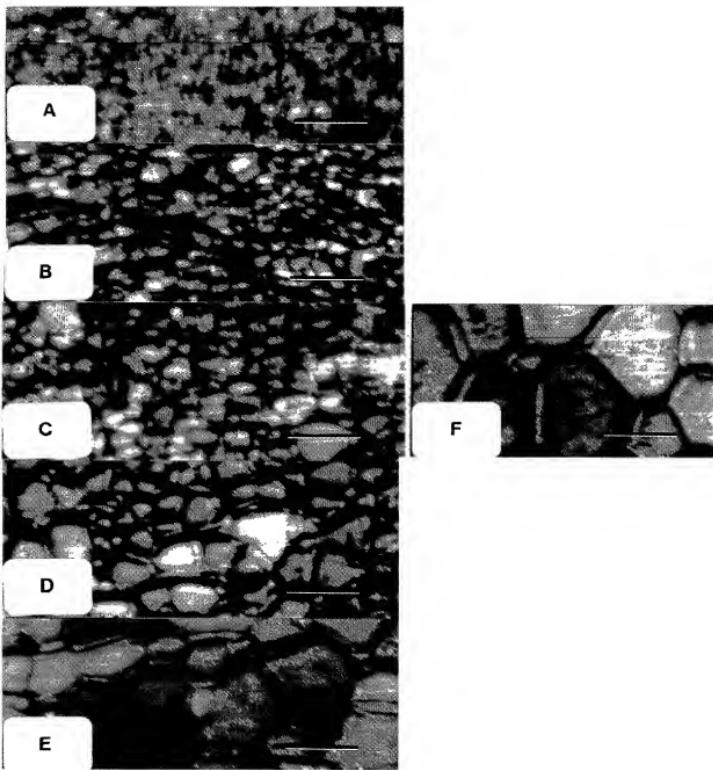


Fig. 8.12

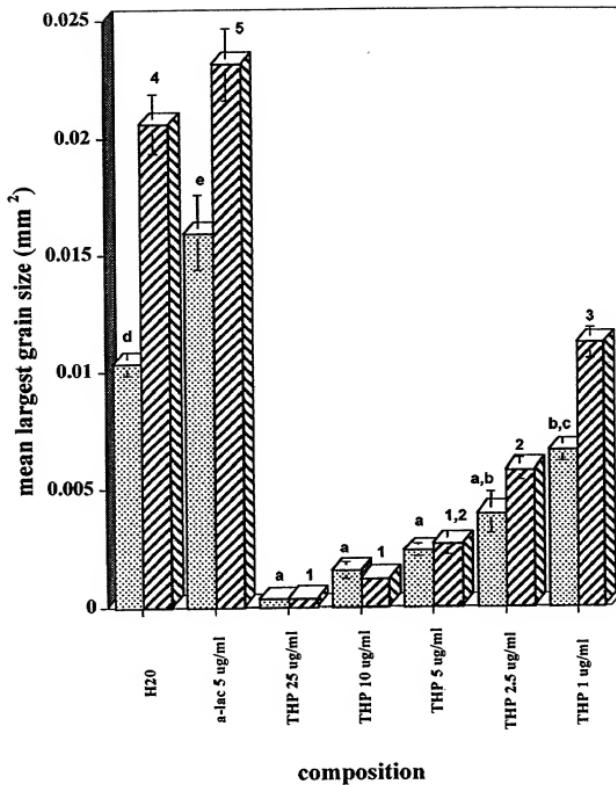


Fig. 8.13

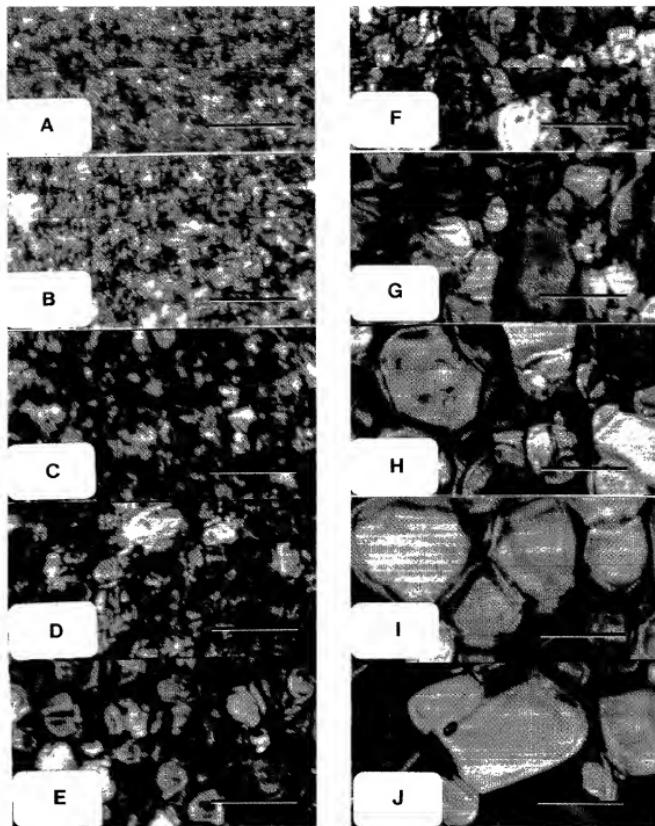


Fig. 8.14

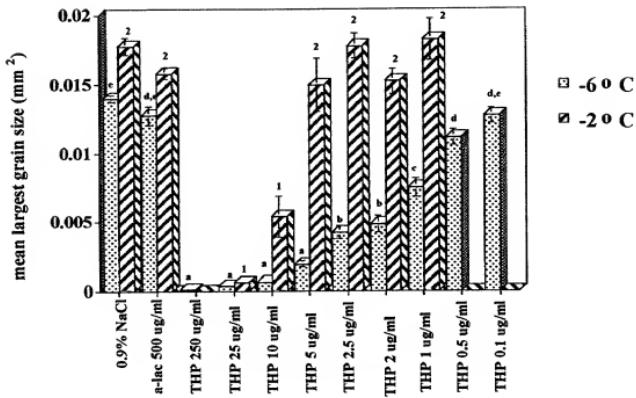


Fig. 8.15

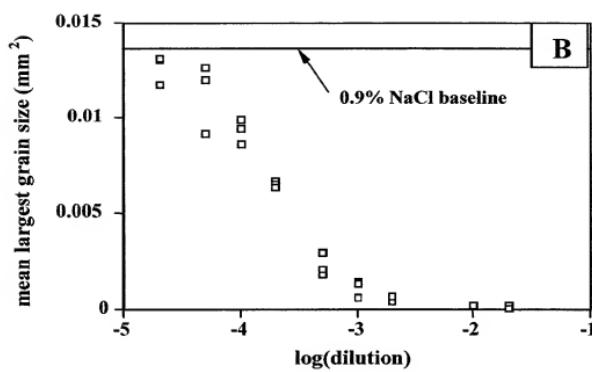
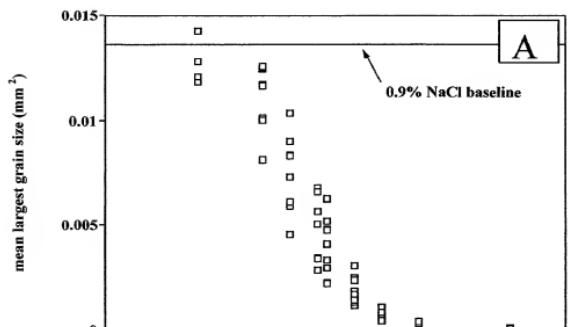


Fig. 8.16

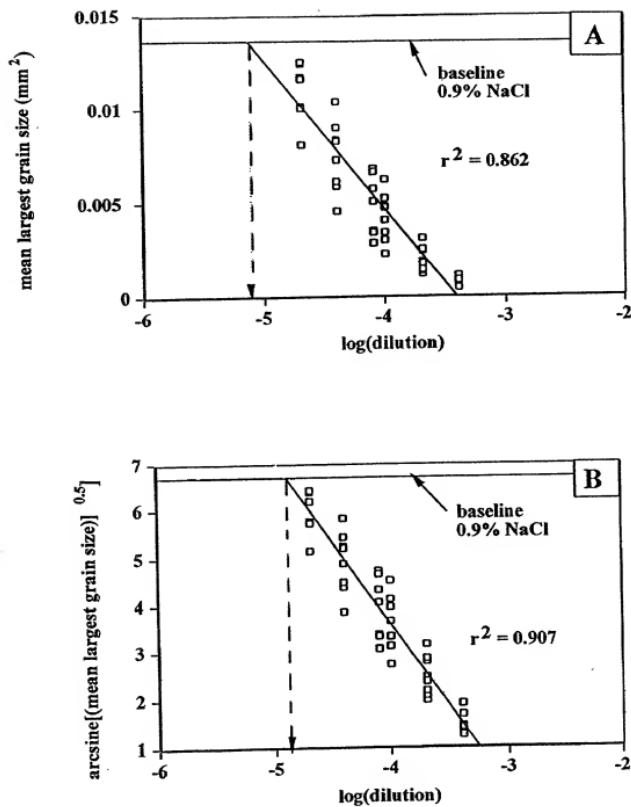


Fig. 8.17

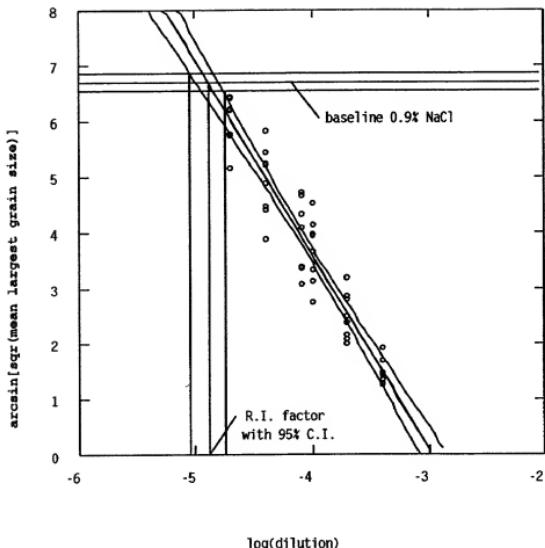


Fig. 8.18

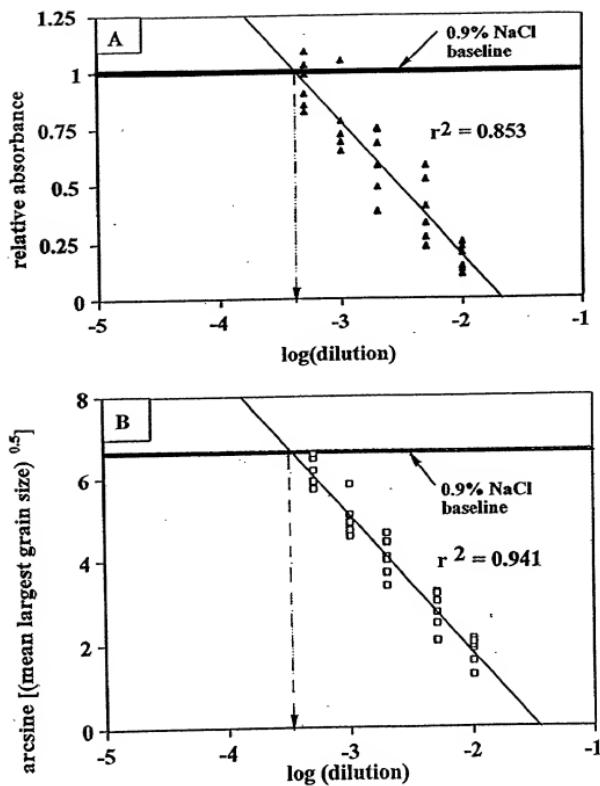


Fig. 8.19

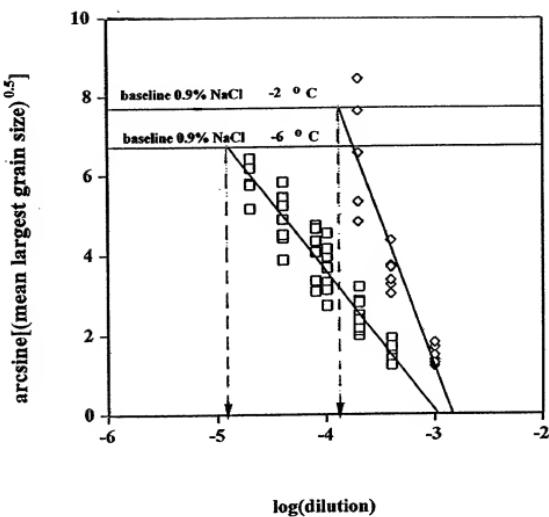


Fig. 8.20

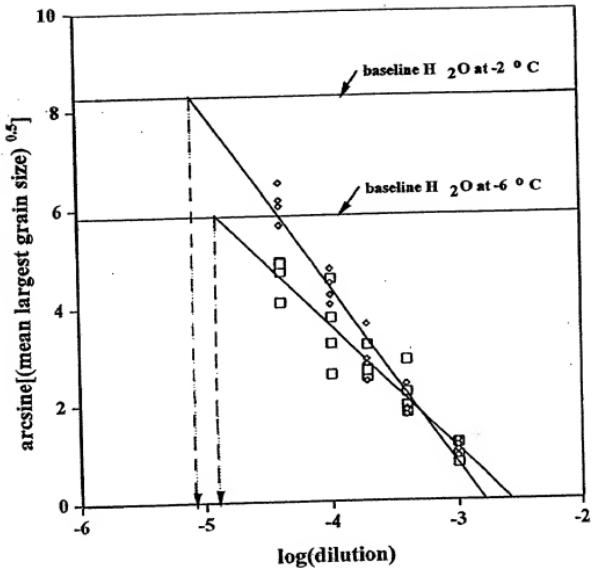


Fig. 8.21

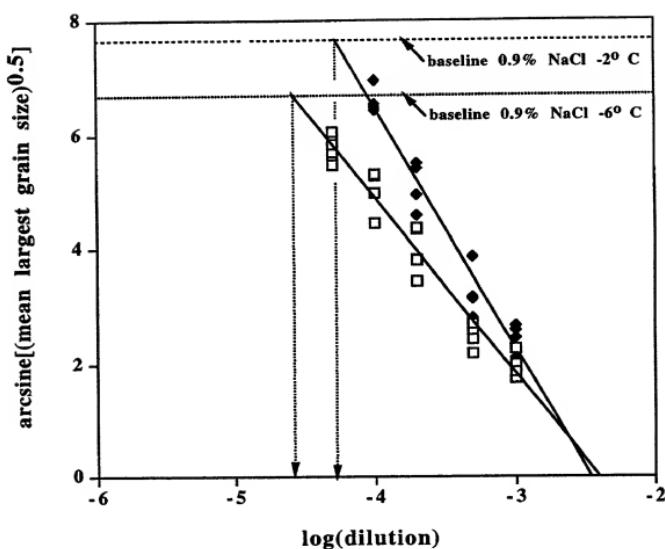


Fig. 8.22

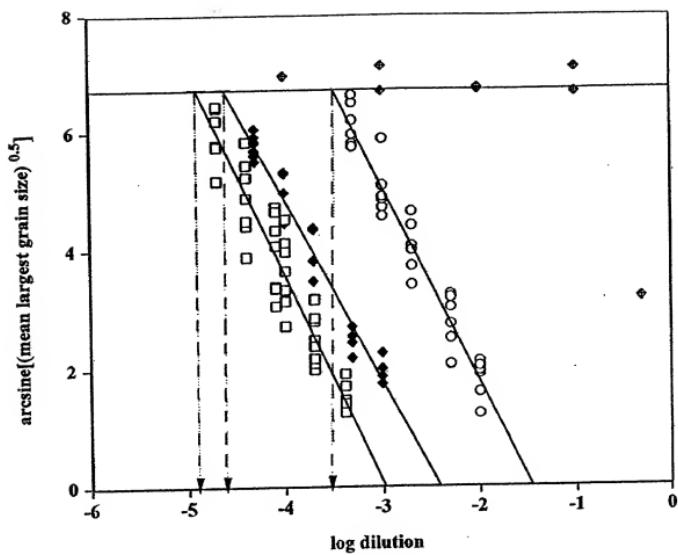


Fig. 8.28

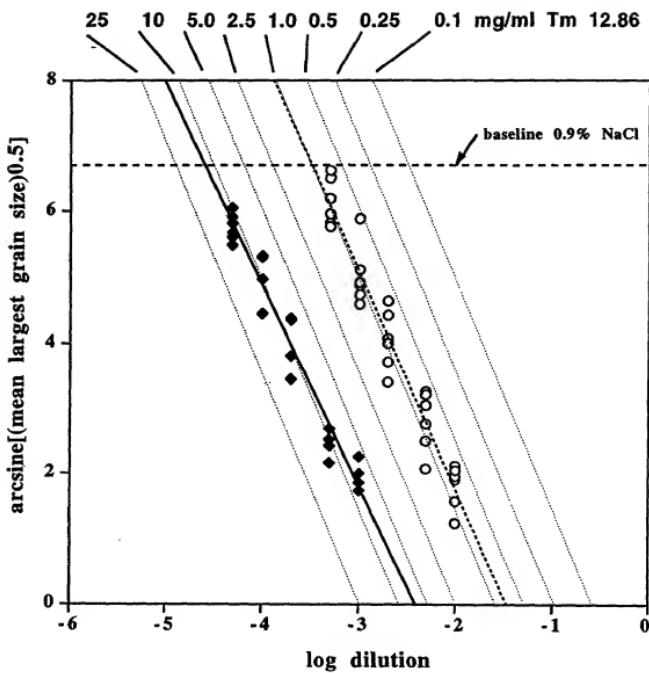


Fig. 8.24

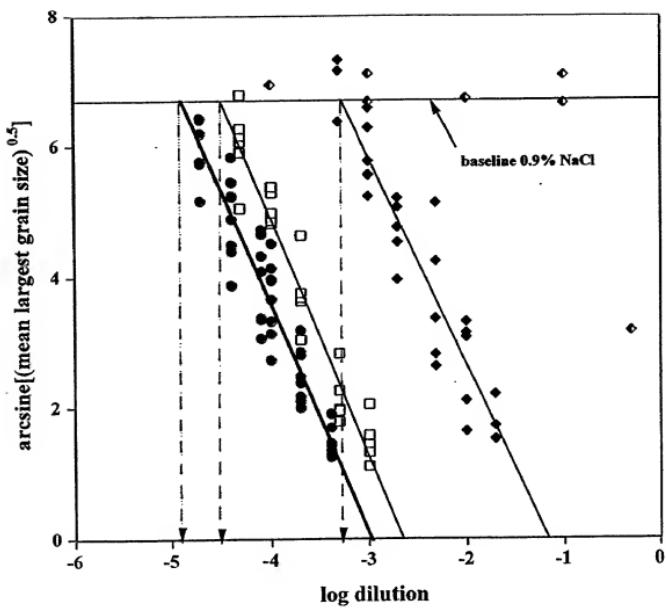


Fig. 8.25

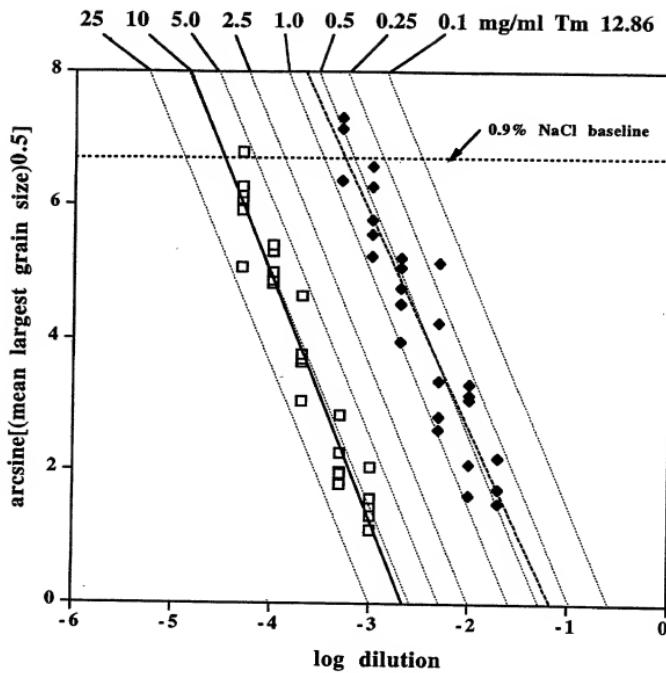


Fig. 8.26

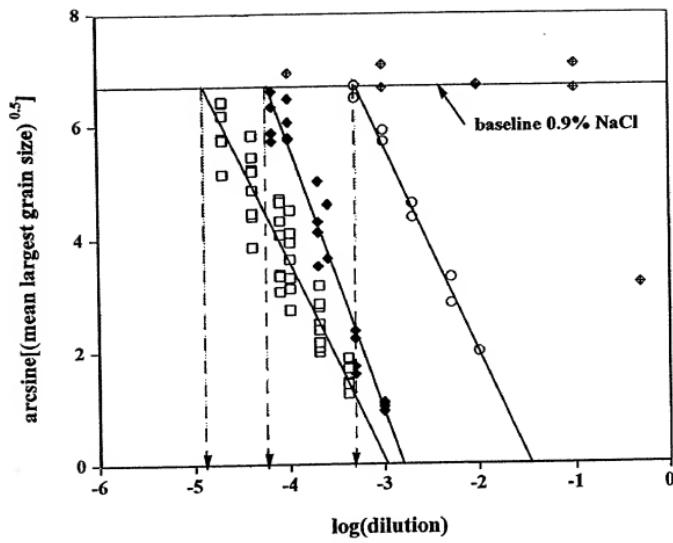


Fig. 8.27

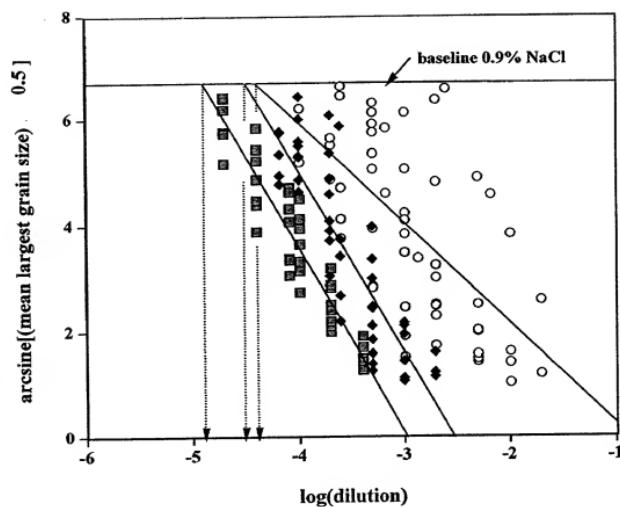


Fig. 8.28

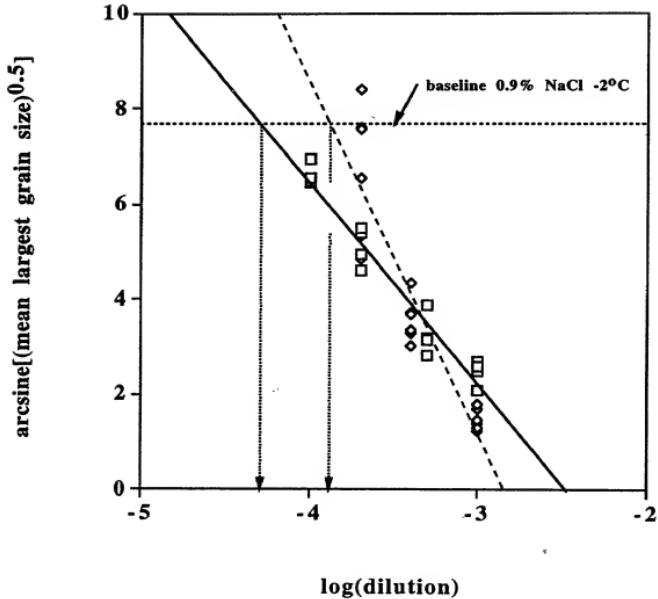


Fig. 8.29

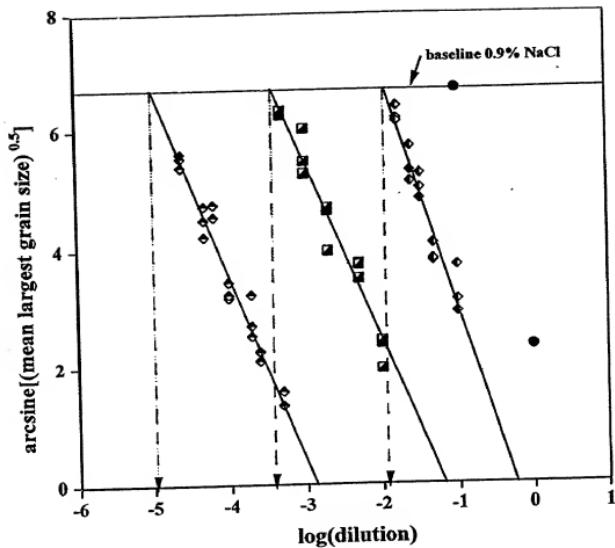


Fig. 8.30

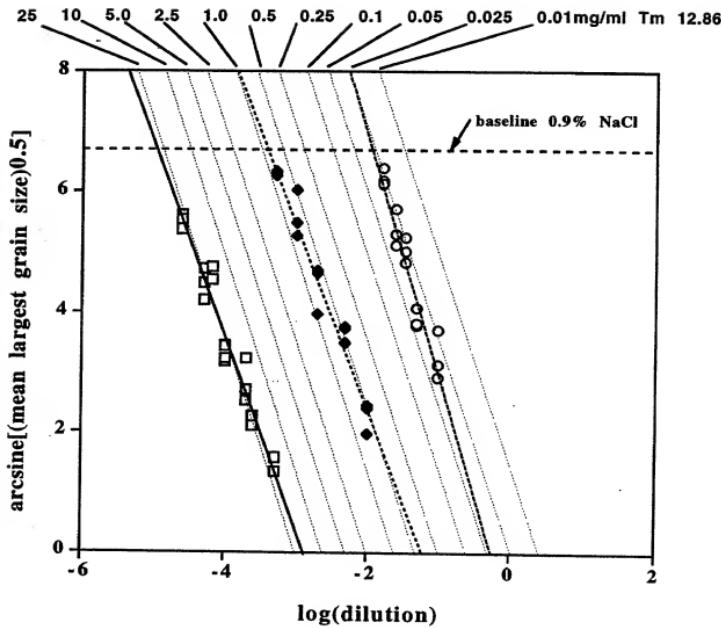


Fig. 8.31

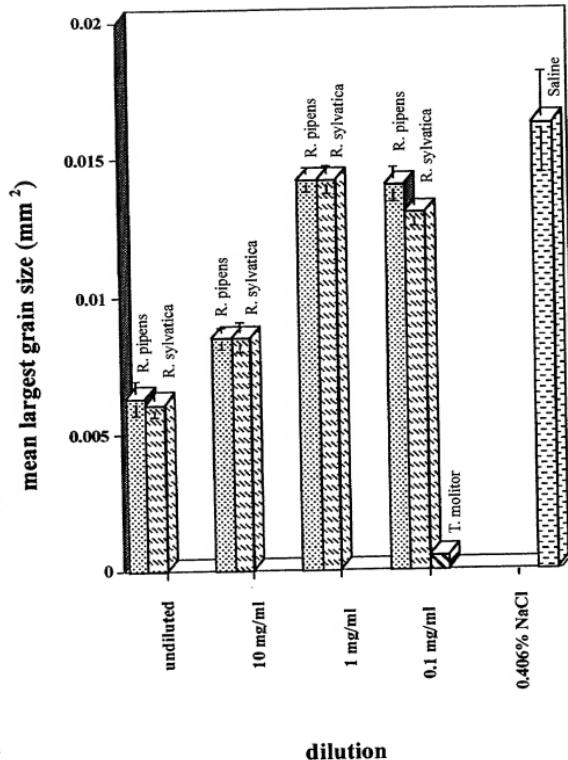


Fig. 8.32

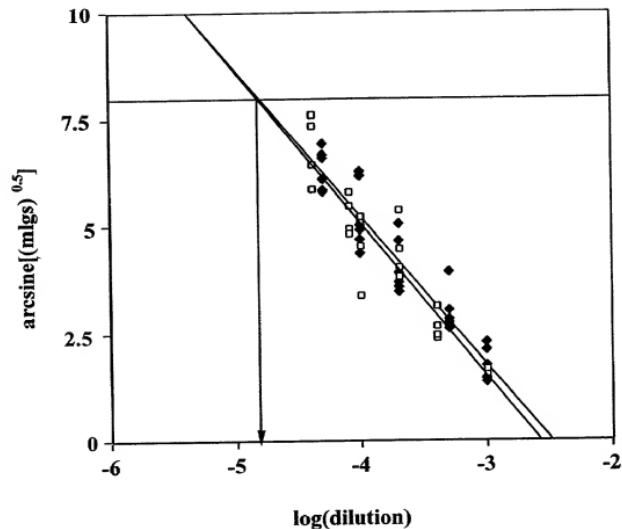


Fig. 8.33

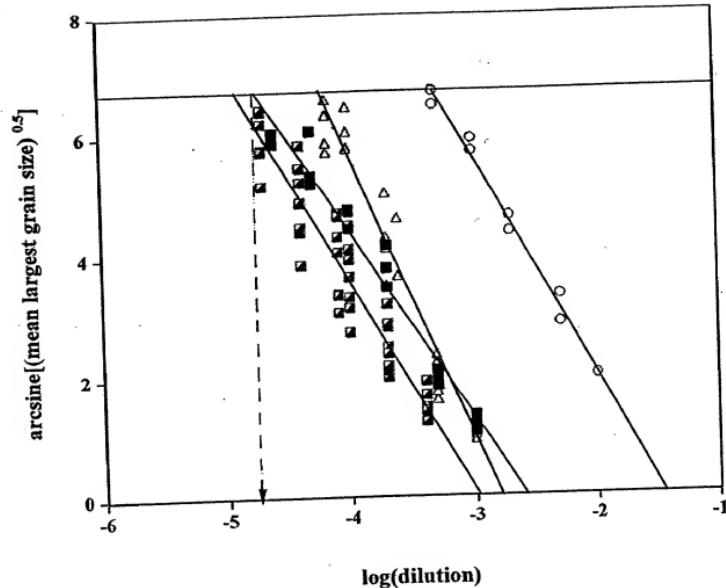


Fig. 8.34

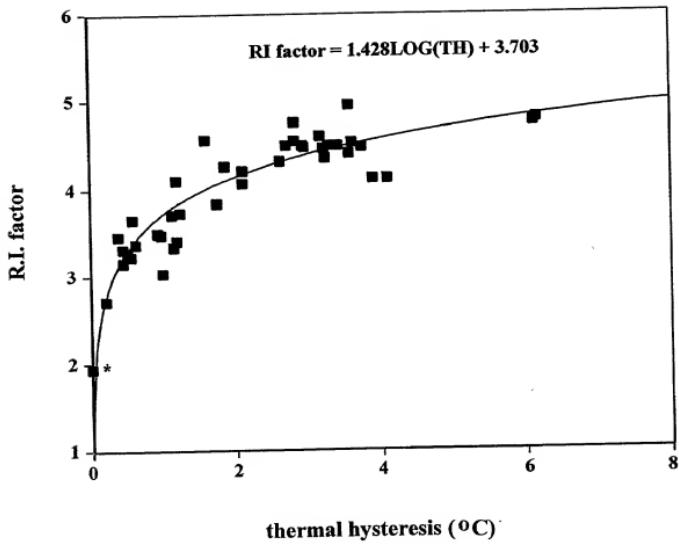


Fig. 8.35

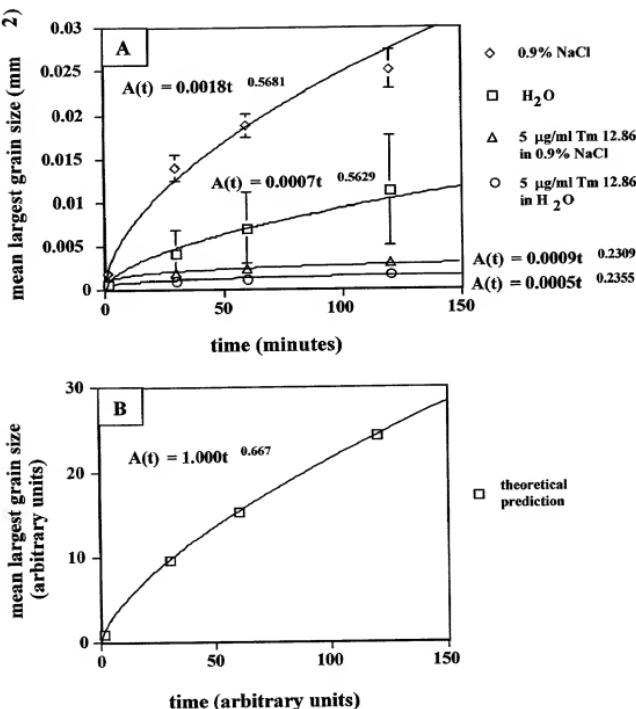


Fig. 8.36

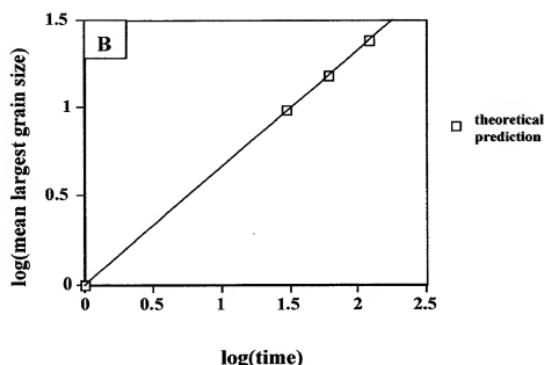
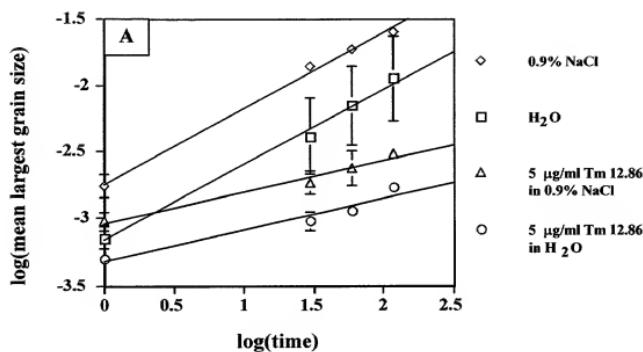
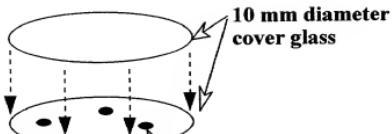


Fig. 8.37

"Sandwich" method of R.I. assessment

1.



2.



**3. FREEZE ON ~80 C
ALUMINUM PLATE (~10 MIN.)**



**4. PLACE ON COLD STAGE,
ANNEAL AT -6 C UP TO
12+ HOURS**

Fig. 8.38

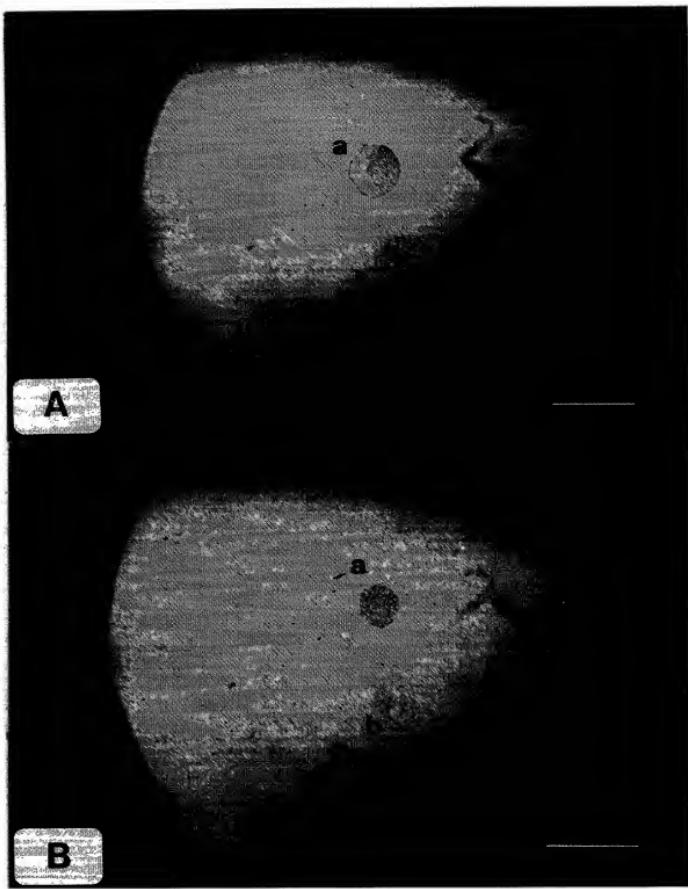


Fig. 8.39

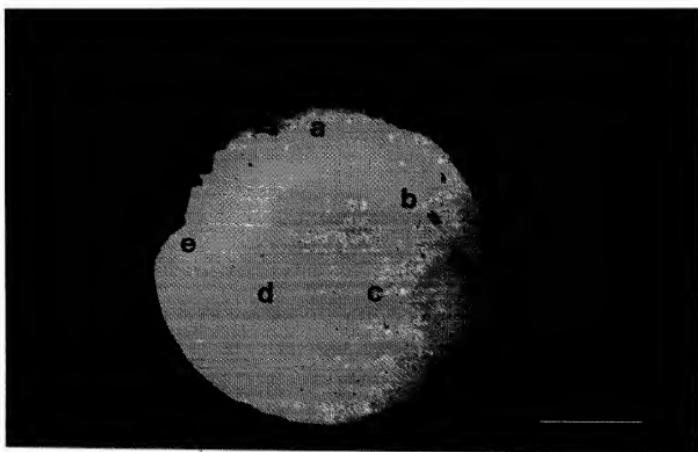


Fig. 8.40

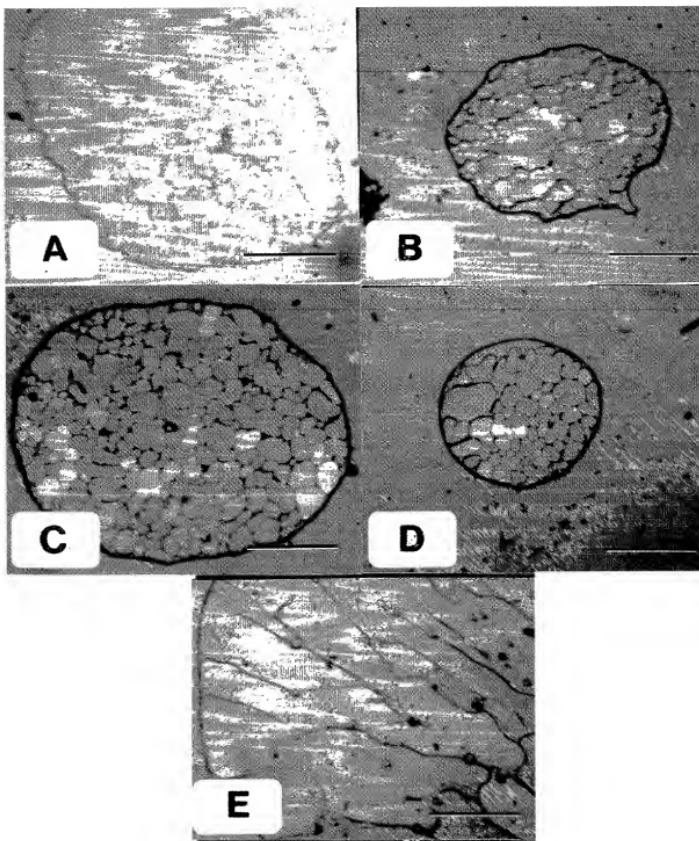


Fig. 8.41

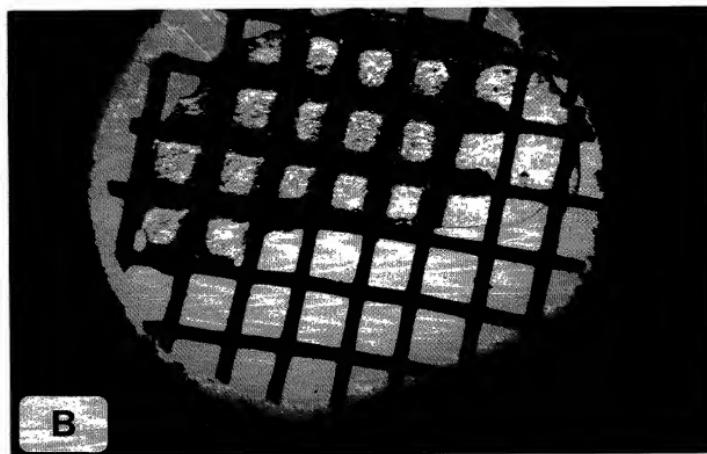
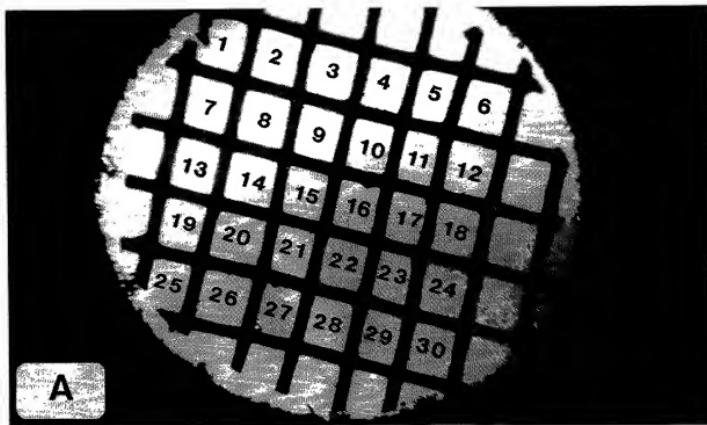


Fig. 8.42

DNA sequence of Tm 13.17 cDNA clone

B E
a c
m o
H R
I I

1 AGTGGATCAAAGAATTGGCACGGAGACTACTAAGATGAAGTTGCTCTGTTGCTAAATCT
M K L L C C C L I S

61 CCCTCATTCTGTTGGTCACAGTTCAAGGCGCTGACCGAGGCACAAATTGAGAAACTGAAAC
L I L L V T V Q A L T E A Q I E K L N K

121 AGATCAGCAAAATGTCAAAATGAAAGTGGAGCTGCGCAAGGATCATAACCAAGCTC
I S K K C Q N E S G V S Q E I I T K A R

181 GCAACGGTCACTGGGAGGACGATCCTAAACTGAAACGCCAAGTTTTGCGTGGCCAGGA
N G D W E D D P K L K R Q V F C V A R N

241 ACGCCGGTCTGGCACGGAATCGGGAGGGTGGTGGTCGACGTGTTGAGGGAGAAGGTGA
A G L A T E S G E V V V D V L R E K V R

301 GGAAGGTCACTGACAACGACGAAGAAACTGAGAAATCATCAATAAGTGCAGCCGTCAGA
K V T D N D E E T E K I I N K C A V K R

361 GAGATACTGTTGAAGAGACGGTGTCAATACTTCAAATGTGTATGAAAAACAGCCAA
D T V E E T V F N T F K C V M K N K P K

421 AGTTCTCACCAAGTTGATTGACCCACCAACGACTAGTAGATGGTCAAATGGTGTGCTTAC
F S P V D *

X
h
o
i

481 ATATAAAATAAAGTGTCTGATGTAaaaaaaaaaaaaaaaaaaaaACTCG
polyadenylation signal poly (A) tail (26)

537 AGAGTATTCTAGAGCGGCCGCGGGCCCATCGTTTCCACCC

Fig. 8.43

P

1 GGCACGAGCAAAATGAAACTCCCTTGTGCTTGCCGTTCGCCGCC
 M K L L L C F A F A A

P G

47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
 I V I G A Q A L T D E Q I Q K

γ

92 AGGAACAAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCC
 R N K I S K E C Q Q V S G V S

γ

137 CAAGAGACGATCGACAAAGTCCCGCACAGGTGTCTTGGTCATGAT
 Q E T I D K V R T G Y L V D D

182 CCCAAATGAAGAACGACGTCCCTGCTTCTCGAAGAAAAACTGGA
 P K M K K H V L C F S K K T G

226 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
 V A T E A G D T N V E V L K A

271 AAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTG
 K L K H V A S D E E V D K I V

P

316 CAGAAGTGCCTGGTCAAGAACGGCCACACCCAGAGGAAACGGCTTAT
 Q K C V V K K A T P E E T A Y

P G

361 GACACCTTCAAGTGTATTTACGACAGCAAACCTGATTTCCTCTCT
 D T F K C I Y D S K P D F S P

G

406 ATTGATTAATTGTTTGTATTTGACTGAATTTGACAATAAAAGT
 I D *

polyadenylation signal

451 ACTATCGTTATGTAAAAAAAAAAAAAAA

poly (A) tail

Fig. 8.44